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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/47, 16/24	A1	(11) International Publication Number: WO 96/12735 (43) International Publication Date: 2 May 1996 (02.05.96)
(21) International Application Number: PCT/US95/12724 (22) International Filing Date: 12 October 1995 (12.10.95) (30) Priority Data: 08/327,514 19 October 1994 (19.10.94) US 08/494,440 19 June 1995 (19.06.95) US 08/533,901 26 September 1995 (26.09.95) US (71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). (72) Inventors: LIN, Lih-Ling; 107 College Road, Concord, MA 01742 (US). CHEN, Jennifer; 833 West Roxbury Parkway, Chestnut Hill, MA 02167 (US). SCHIEVELLA, Andrea, R.; 39 Edward Street, Winchester, MA 01890 (US). GRAHAM, James; 28 Foscett Street, Somerville, MA 02144 (US). (74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).		(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> BEST AVAILABLE COPY
(54) Title: NOVEL TNF RECEPTOR DEATH DOMAIN LIGAND PROTEINS AND INHIBITORS OF LIGAND BINDING (57) Abstract Novel TNF receptor death domain ("TNF-R1-DD") ligand proteins are disclosed. Polynucleotides encoding the TNF-R1-DD ligand protein are also disclosed, along with vectors, host cells, and methods of making the TNF-R1-DD ligand protein. Pharmaceutical compositions containing the TNF-R1-DD ligand protein, methods of treating inflammatory conditions, and methods of inhibiting TNF-R death domain binding are also disclosed. Methods of identifying inhibitors of TNF-R death domain binding and inhibitors identified by such methods are also disclosed.		

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NOVEL TNF RECEPTOR
DEATH DOMAIN LIGAND PROTEINS
AND INHIBITORS OF LIGAND BINDING

This application is a continuation-in-part of application Ser. No. 08/494,440, filed June 19, 1995, which was a continuation-in-part of application Ser. No. 08/327,514, filed October 19, 1994.

10

BACKGROUND OF THE INVENTION

The present invention relates to the field of anti-inflammatory substances and other substances which act by inhibiting binding to the intracellular domain of a tumor necrosis factor receptor (hereinafter "TNF-R"), such as, for example, the P55 type (or TNF-R1) TNF receptor. More particularly, the present invention is directed to novel ligands which bind to the TNF-R intracellular domain and to inhibition or modulation of signal transduction by this receptor.

Tumor necrosis factor (herein "TNF") is a cytokine which produces a wide range of cellular activities. TNF causes an inflammatory response, which can be beneficial, such as in mounting an immune response to a pathogen, or when overexpressed can lead to other detrimental effects of inflammation.

The cellular effects of TNF are initiated by the binding of TNF to its receptors (TNF-Rs) on the surface of target cells. The isolation of polynucleotides encoding TNF-Rs and variant forms of such receptors has been described in European patent publication Nos. EP 308,378, EP 393,438, EP 433,900, EP 526,905 and EP 568,925; in PCT patent publication Nos. WO91/03553 and WO93/19777; and by Schall *et al.*, Cell 61:361-370 (1990) (disclosing the P55 type TNF receptor). Processes for purification of TNF-Rs have also been disclosed in U.S. Patent No. 5,296,592.

Native TNF-Rs are characterized by distinct extracellular, transmembrane and intracellular domains. The primary purpose of the extracellular domain is to present a binding site for TNF on the outside of the cell. When TNF is bound to the binding site, a "signal" is transmitted to the inside of the cell through

the transmembrane and intracellular domains, indicating that binding has occurred. Transmission or "transduction" of the signal to the inside of the cell occurs by a change in conformation of the transmembrane and/or intracellular domains of the receptor. This signal is "received" by the binding of proteins and other molecules to the intracellular domain of the receptor, resulting in the effects seen upon TNF stimulation. Two distinct TNF receptors of ~55 kd ("TNF-R1") and ~75 kd ("TNF-R2") have been identified. Numerous studies with anti-TNF receptor antibodies have demonstrated that TNF-R1 is the receptor which signals the majority of the pleiotropic activities of TNF. Recently, the domain required for signaling cytotoxicity and other TNF-mediated responses has been mapped to the ~80 amino acid near the C-terminus of TNF-R1. This domain is therefore termed the "death domain" (hereinafter referred to as "TNF-R death domain" and "TNF-R1-DD") (see Tartaglia *et al.*, Cell 74:845-853 (1993)).

While TNF binding by TNF-Rs results in beneficial cellular effects, it is often desirable to prevent or deter TNF binding from causing other detrimental cellular effects. Although substantial effort has been expended investigating inhibition of TNF binding to the extracellular domain of TNF-Rs, examination of binding of proteins and other molecules to the intracellular domain of TNF-Rs has received much less attention.

However, ligands which bind to the TNF-R intracellular domain have yet to be identified. It would be desirable to identify and isolate such ligands to examine their effects upon TNF-R signal transduction and their use as therapeutic agents for treatment of TNF-induced conditions. Furthermore, identification of such ligands would provide a means for screening for inhibitors of TNF-R/intracellular ligand binding, which will also be useful as anti-inflammatory agents.

SUMMARY OF THE INVENTION

Applicants have for the first time identified novel TNF-R1-DD ligand proteins and have isolated polynucleotides encoding such ligands. Applicants have also identified a known protein which may also bind to the death domain of TNF-R.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide encoding a protein having TNF-R1-DD ligand

protein activity. In preferred embodiments, the polynucleotide is selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;
- 5 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1;
- (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;
- (d) a polynucleotide encoding an TNF-R1-DD ligand protein
10 comprising a fragment of the amino acid sequence of SEQ ID NO:2;
- (e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;
- (f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3;
- 15 (g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;
- (h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4;
- (i) a polynucleotide comprising the nucleotide sequence of SEQ
20 ID NO:9 from nucleotide 2 to nucleotide 931;
- (j) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9;
- (k) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;
- 25 (l) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10;
- (m) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;
- (n) a polynucleotide comprising a fragment of the nucleotide
30 sequence of SEQ ID NO:11;
- (o) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12;

(p) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12;

(q) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 3 to nucleotide 2846;

5 (r) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13, which encodes a protein having TNF-R1-DD ligand protein activity;

(s) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;

10 (t) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 and having TNF-R1-DD ligand protein activity; and

(u) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(t).

15 In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions.

Processes are also provided for producing an TNF-R1-DD ligand protein.
20 which comprises:

(a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and

(b) purifying the TNF-R1-DD ligand protein from the culture.

The ligand protein produced according to such methods is also provided by the
25 present invention.

Compositions comprising a protein having TNF-R1-DD ligand protein activity are also disclosed. In preferred embodiments the protein comprises an amino acid sequence selected from the group consisting of:

- 30 (a) the amino acid sequence of SEQ ID NO:2;
(b) fragments of the amino acid sequence of SEQ ID NO:2;
(c) the amino acid sequence of SEQ ID NO:4;
(d) fragments of the amino acid sequence of SEQ ID NO:4;

- (e) the amino acid sequence of SEQ ID NO:6;
- (f) fragments of the amino acid sequence of SEQ ID NO:6;
- (g) the amino acid sequence of SEQ ID NO:10;
- (h) fragments of the amino acid sequence of SEQ ID NO:10;
- 5 (i) the amino acid sequence of SEQ ID NO:12;
- (j) fragments of the amino acid sequence of SEQ ID NO:12;
- (k) the amino acid sequence of SEQ ID NO:14; and
- (l) fragments of the amino acid sequence of SEQ ID NO:14;

the protein being substantially free from other mammalian proteins. Such
10 compositions may further comprise a pharmaceutically acceptable carrier.

Compositions comprising an antibody which specifically reacts with
such TNF-R1-DD ligand protein are also provided by the present invention.

Methods are also provided for identifying an inhibitor of TNF-R death
domain binding which comprise:

- 15 (a) combining an TNF-R death domain protein with an TNF-R1-DD ligand protein, said combination forming a first binding mixture;
- (b) measuring the amount of binding between the TNF-R death domain protein and the TNF-R1-DD ligand protein in the first binding mixture;
- 20 (c) combining a compound with the TNF-R death domain protein and an TNF-R1-DD ligand protein to form a second binding mixture;
- (d) measuring the amount of binding in the second binding mixture; and
- (e) comparing the amount of binding in the first binding mixture
25 with the amount of binding in the second binding mixture;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the amount of binding of the second binding mixture occurs. In certain preferred embodiments the TNF-R1-DD ligand protein used in such method comprises an amino acid sequence selected from the group consisting of:

- 30 (a) the amino acid sequence of SEQ ID NO:2;
- (b) fragments of the amino acid sequence of SEQ ID NO:2;
- (c) the amino acid sequence of SEQ ID NO:4;

- (d) fragments of the amino acid sequence of SEQ ID NO:4;
- (e) the amino acid sequence of SEQ ID NO:6;
- (f) fragments of the amino acid sequence of SEQ ID NO:6;
- (g) the amino acid sequence of SEQ ID NO:8;
- 5 (h) fragments of the amino acid sequence of SEQ ID NO:8
- (i) the amino acid sequence of SEQ ID NO:10;
- (j) fragments of the amino acid sequence of SEQ ID NO:10;
- (k) the amino acid sequence of SEQ ID NO:12;
- (l) fragments of the amino acid sequence of SEQ ID NO:12;
- 10 (m) the amino acid sequence of SEQ ID NO:14; and
- (n) fragments of the amino acid sequence of SEQ ID NO:14.

Compositions comprising inhibitors identified according to such method are also provided. Such compositions may include pharmaceutically acceptable carriers.

15 Methods are also provided for preventing or ameliorating an inflammatory condition which comprises administering a therapeutically effective amount of a composition comprising a protein having TNF-R1-DD ligand protein activity and a pharmaceutically acceptable carrier.

Other embodiments provide methods of inhibiting TNF-R death domain binding comprising administering a therapeutically effective amount of a
20 composition comprising a protein having TNF-R1-DD ligand protein activity and a pharmaceutically acceptable carrier.

Methods are also provided for preventing or ameliorating an inflammatory condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a pharmaceutically
25 acceptable carrier and a protein selected from the group consisting of insulin-like growth factor binding protein-5 ("IGFBP-5"), and fragments thereof having TNF-R1-DD ligand protein activity. Such proteins may also be administered for inhibiting TNF-R death domain binding.

30 Methods of preventing or ameliorating an inflammatory condition or of inhibiting TNF-R death domain binding are provided, which comprise administering to a mammalian subject a therapeutically effective amount of inhibitors of TNF-R death domain binding, are also provided.

Methods of identifying an inhibitor of TNF-R death domain binding are also provided by the present invention which comprise:

5 (a) transforming a cell with a first polynucleotide encoding an TNF-R death domain protein, a second polynucleotide encoding an TNF-R1-DD ligand protein, and at least one reporter gene, wherein the expression of the reporter gene is regulated by the binding of the TNF-R1-DD ligand protein encoded by the second polynucleotide to the TNF-R death domain protein encoded by the first polynucleotide;

10 (b) growing the cell in the presence of and in the absence of a compound; and

(c) comparing the degree of expression of the reporter gene in the presence of and in the absence of the compound;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the degree of expression of the reporter gene occurs. In preferred
15 embodiments, the cell is a yeast cell and the second polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;

20 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1, which encodes a protein having TNF-R1-DD ligand protein activity;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;

25 (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 and having TNF-R1-DD ligand protein activity;

(e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;

30 (f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3, which encodes a protein having TNF-R1-DD ligand protein activity;

- (g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;
- (h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 and having TNF-R1-DD ligand protein activity;
- 5 (i) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 2 to nucleotide 559;
- (j) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5, which encodes a protein having TNF-R1-DD ligand protein activity;
- 10 (k) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:6;
- (l) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 and having TNF-R1-DD ligand protein activity;
- 15 (m) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 57 to nucleotide 875;
- (n) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7, which encodes a protein having TNF-R1-DD ligand protein activity;
- 20 (o) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:8;
- (p) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 and having TNF-R1-DD ligand protein activity;
- 25 (q) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;
- (r) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9;
- 30 (s) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(t) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10;

(u) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;

5 (v) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11;

(w) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12;

10 (x) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12;

(y) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 3 to nucleotide 2846;

15 (z) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13, which encodes a protein having TNF-R1-DD ligand protein activity;

(aa) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;

20 (bb) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 and having TNF-R1-DD ligand protein activity; and

(cc) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(bb). which encodes a protein having TNF-R1-DD ligand protein activity.

25

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1 and 2 depict autoradiographs demonstrating the expression of TNF-R1-DD ligand proteins of the present invention.

Fig. 3 depicts an autoradiograph demonstrating the expression of clones 1TU, 15TU and 27TU.

30

Fig. 4 demonstrates the binding of 1TU and 27TU to TNF-R1-DD. MBP, MBP-1TU or MBP-27TU (3 μ g) was incubated with glutathione beads containing 3 μ g of either GST or GST-TNF-R1-DD in 100 μ l of binding buffer (0.2%

Triton, 20 mM Tris pH 7.5, 140 mM NaCl, 0.1 mM EDTA, 10 mM DTT and 5% glycerol). The reaction was performed at 4°C for 2 hours and centrifuged to remove unbound fraction (Unbound). The beads were then washed with 500µl binding buffer four times and resuspended into SDS-sample buffer (Bound). These samples
5 were analyzed by Western blot using anti-MBP antibody (New England Biolab).

Fig. 5 demonstrates the ability of 15TU and 27TU to activate the JNK pathway. COS cells were cotransfected with HA-tagged JNK1 and clones 15tu or 27TU. Cells were left untreated or treated for 15 min with 50 ng/ml TNF, and HA-JNK1 was immunoprecipitated with anti-HA antibody. JNK activity was measured
10 in an *in vitro* kinase assay using GST-c-jun (amino acids 1-79) as substrate, and reactions were electrophoresed on SDS-PAGE.

Fig. 6 is an autoradiograph of an SDS-PAGE gel of conditioned media from COS cells transfected with clone 3TW.

Fig. 7 is an autoradiograph which demonstrates that an antisense
15 oligonucleotide derived from the sequence of clone 3TW inhibits TNF-induced cPLA₂ phosphorylation.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have for the first time identified and isolated
20 novel polynucleotides which encode proteins which bind to the TNF-R death domain. As used herein "TNF-R" includes all receptors for tumor necrosis factor. The P55 type TNF-R is the preferred receptor for practicing the present invention.

The sequence of a polynucleotide encoding one such protein is set forth in SEQ ID NO:1 from nucleotides 2 to 1231. This polynucleotide has been
25 identified as "clone 2DD". The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 2DD is set forth in SEQ ID NO:2. It is believed that clone 2DD is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 2DD does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone
30 2DD was deposited with the American Type Culture Collection on October 13, 1994 and given the accession number ATCC 69706.

The protein encoded by clone 2DD is 410 amino acids in length. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA searches. Therefore, clone 2DD encodes a novel protein.

5 The sequence of a polynucleotide encoding one such protein is set forth in SEQ ID NO:3 from nucleotides 2 to 415. This polynucleotide has been identified as "clone 3DD". The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 3DD is set forth in SEQ ID NO:4. It is believed that clone 3DD is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 3DD does bind the death domain
10 of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone 3DD was deposited with the American Type Culture Collection on October 13, 1994 and given the accession number ATCC 69705.

The protein encoded by clone 3DD is 138 amino acids. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA
15 searches. Therefore, clone 3DD encodes a novel protein.

A full-length clone corresponding to clone 3DD was also isolated and identified as "clone 3TW". The nucleotide sequence of clone 3TW is reported as SEQ ID NO:13. Nucleotides 3 to 2846 of SEQ ID NO:13 encode a TNF-R1-DD ligand protein, the amino acid sequence of which is reported as SEQ ID NO:14.
20 Amino acids 811 to 948 of SEQ ID NO:14 correspond to amino acids 1 to 138 of SEQ ID NO:4 (clone 3DD). Clone 3TW was deposited with the American Type Culture Collection on September 26, 1995 and given the accession number ATCC

The sequence of a polynucleotide encoding another such protein is set forth in SEQ ID NO:5 from nucleotides 2 to 559. This polynucleotide has been identified as "clone 20DD." The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 20DD is set forth in SEQ ID NO:6. It is believed that clone 20DD is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 20DD does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined
30 herein). Clone 20DD was deposited with the American Type Culture Collection on October 13, 1994 and given the accession number ATCC 69704.

The protein encoded by clone 20DD is identical to amino acids 87 to 272 of insulin-like growth factor binding protein-5 ("IGFBP-5"), a sequence for which was disclosed in J. Biol. Chem. 266:10646-10653 (1991) by Shimasaki *et al.*, which is incorporated herein by reference. The polynucleotide and amino acid sequences of IGFBP-5 are set forth in SEQ ID NO:7 and SEQ ID NO:8, respectively. Based upon the sequence identity between clone 20DD and IGFBP-5, IGFBP-5 and certain fragments thereof will exhibit TNF-R1-DD ligand binding activity (as defined herein).

The sequence of a polynucleotide encoding another such protein is set forth in SEQ ID NO:9 from nucleotides 2 to 931. This polynucleotide has been identified as "clone 1TU". The amino-acid sequence of the TNF-R1-DD ligand protein encoded by clone 1TU is set forth in SEQ ID NO:10. It is believed that clone 1TU is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 1TU does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone 1TU was deposited with the American Type Culture Collection on June 7, 1995 and given the accession number ATCC 69848.

The protein encoded by clone 1TU is 310 amino acids in length. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA searches. Therefore, clone 1TU encodes a novel protein.

The sequence of a polynucleotide encoding another such protein is set forth in SEQ ID NO:11 from nucleotides 2 to 1822. This polynucleotide has been identified as "clone 27TU". The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 27TU is set forth in SEQ ID NO:12. It is believed that clone 27TU is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 27TU does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone 27TU was deposited with the American Type Culture Collection on June 7, 1995 and given the accession number ATCC 69846.

The protein encoded by clone 27TU is 607 amino acids in length. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA searches. Therefore, clone 27TU encodes a novel protein. 27TU may be

a longer version of clone 2DD. 2DD encodes the same amino acid sequence (SEQ ID NO:2) as amino acids 198-607 encoded by 27TU (SEQ ID NO:12). The nucleotide sequences of 2DD and 27TU are also identical within this region of identity.

5 An additional "clone 15TU" was isolated which encoded a portion of the 27TU sequence (approximately amino acids 289-607 of SEQ ID NO:12). Clone 15TU was deposited with the American Type Culture Collection on June 7, 1995 and given the accession number ATCC 69847. 15TU comprises the same nucleotide sequence as 27TU over this region of amino acids.

10 Polynucleotides hybridizing to the polynucleotides of the present invention under stringent conditions and highly stringent conditions are also part of the present invention. As used herein, "highly stringent conditions" include, for example, 0.2xSSC at 65°C; and "stringent conditions" include, for example, 4xSSC at 65°C or 50% formamide and 4xSSC at 42°C.

15 For the purposes of the present application, "TNF-R1-DD ligand protein" includes proteins which exhibit TNF-R1-DD ligand protein activity. For the purposes of the present application, a protein is defined as having "TNF-R1-DD ligand protein activity" when it binds to a protein derived from the TNF-R death domain. Activity can be measured by using any assay which will detect binding to
20 an TNF-R death domain protein. Examples of such assays include without limitation the interaction trap assays and assays in which TNF-R death domain protein which is affixed to a surface in a manner conducive to observing binding, including without limitation those described in Examples 1 and 3. As used herein an "TNF-R death domain protein" includes the entire death domain or fragments thereof.

25 Fragments of the TNF-R1-DD ligand protein which are capable of interacting with the TNF-R death domain or which are capable of inhibiting TNF-R death domain binding (i.e., exhibit TNF-R1-DD ligand protein activity) are also encompassed by the present invention. Fragments of the TNF-R1-DD ligand protein may be in linear form or they may be cyclized using known methods, for example,
30 as described in H.U. Saragovi, *et al.*, *Bio/Technology* 10, 773-778 (1992) and in R.S. McDowell, *et al.*, *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules

such as immunoglobulins for many purposes, including increasing the valency of TNF-R1-DD ligand protein binding sites. For example, fragments of the TNF-R1-DD ligand protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the TNF-R1-DD ligand protein, such a
5 fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, an TNF-R1-DD ligand protein - IgM fusion would generate a decavalent form of the TNF-R1-DD ligand protein of the invention.

The isolated polynucleotide of the invention may be operably linked
10 to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the TNF-R1-DD ligand protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology
15 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and the expression control sequence are situated within a vector or cell in such a way that the TNF-R1-DD ligand protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

20 A number of types of cells may act as suitable host cells for expression of the TNF-R1-DD ligand protein. Host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro
25 culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

The TNF-R1-DD ligand protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system.
30 Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described

in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference.

Alternatively, it may be possible to produce the TNF-R1-DD ligand protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*,
5 *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the TNF-R1-DD ligand protein
10 is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional TNF-R1-DD ligand protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The TNF-R1-DD ligand protein of the invention may also be
15 expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the TNF-R1-DD ligand protein.

The TNF-R1-DD ligand protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the
20 recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the TNF-R1-DD ligand protein may also include an affinity column containing the TNF-R death domain or other TNF-R death domain protein; one or more column
25 steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the TNF-R1-DD ligand protein of the invention may
30 also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP) or glutathione-S-transferase (GST). Kits for expression and purification of such fusion

proteins are commercially available from New England BioLab (Beverly, MA) and Pharmacia (Piscataway, NJ), respectively. The TNF-R ligand protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from
5 Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the TNF-R1-DD ligand protein. Some or all of the foregoing
10 purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The TNF-R1-DD ligand protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated TNF-R1-DD ligand protein."

TNF-R1-DD ligand proteins may also be produced by known
15 conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with TNF-R1-DD ligand proteins may possess biological properties in common therewith, including TNF-R1-
20 DD ligand protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified TNF-R1-DD ligand proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The TNF-R1-DD ligand proteins provided herein also include proteins
25 characterized by amino acid sequences similar to those of purified TNF-R1-DD ligand proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the TNF-R1-DD ligand protein sequences may include the replacement, insertion
30 or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Mutagenic techniques for such

replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584).

Other fragments and derivatives of the sequences of TNF-R1-DD ligand proteins which would be expected to retain TNF-R1-DD ligand protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

TNF-R1-DD ligand protein of the invention may also be used to screen for agents which are capable of inhibiting or blocking binding of an TNF-R1-DD ligand protein to the death-domain of TNF-R, and thus may act as inhibitors of TNF-R death domain binding and/or TNF activity. Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the TNF-R1-DD ligand protein of the invention. Examples 1 and 3 describe examples of such assays. Appropriate screening assays may be cell-based or cell-free. Alternatively, purified protein based screening assays may be used to identify such agents. For example, TNF-R1-DD ligand protein may be immobilized in purified form on a carrier and binding to purified TNF-R death domain may be measured in the presence and in the absence of potential inhibiting agents. A suitable binding assay may alternatively employ purified TNF-R death domain immobilized on a carrier, with a soluble form of a TNF-R1-DD ligand protein of the invention. Any TNF-R1-DD ligand protein may be used in the screening assays described above.

In such a screening assay, a first binding mixture is formed by combining TNF-R death domain protein and TNF-R1-DD ligand protein, and the amount of binding in the first binding mixture (B_0) is measured. A second binding mixture is also formed by combining TNF-R death domain protein, TNF-R1-DD ligand protein, and the compound or agent to be screened, and the amount of binding in the second binding mixture (B) is measured. The amounts of binding in the first and second binding mixtures are compared, for example, by performing a B/B_0 calculation. A compound or agent is considered to be capable of inhibiting TNF-R death domain binding if a decrease in binding in the second binding mixture as

compared to the first binding mixture is observed. The formulation and optimization of binding mixtures is within the level of skill in the art. Such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding, and additional control assays may be included in the screening assay of the invention.

5 Alternatively, appropriate screening assays may be cell based. For example, the binding or interaction between an TNF-R ligand protein and the TNF-R death domain can be measured in yeast as described below in Examples 1 and 3.

Compounds found to reduce, preferably by at least about 10%, more preferably greater than about 50% or more, the binding activity of TNF-R1-DD ligand protein to TNF-R death domain may thus be identified and then secondarily
10 screened in other binding assays, including *in vivo* assays. By these means compounds having inhibitory activity for TNF-R death domain binding which may be suitable as anti-inflammatory agents may be identified.

Isolated TNF-R1-DD ligand protein may be useful in treating,
15 preventing or ameliorating inflammatory conditions and other conditions, such as cachexia, autoimmune disease, graft versus host reaction, osteoporosis, colitis, myelogenous leukemia, diabetes, wasting, and atherosclerosis. Isolated TNF-R1-DD ligand protein may be used itself as an inhibitor of TNF-R death domain binding or to design inhibitors of TNF-R death domain binding. Inhibitors of binding of TNF-
20 R1-DD ligand protein to the TNF-R death domain ("TNF-R intracellular binding inhibitors") are also useful for treating such conditions.

The present invention encompasses both pharmaceutical compositions and therapeutic methods of treatment or use which employ isolated TNF-R1-DD ligand protein and/or binding inhibitors of TNF-R intracellular binding.

25 Isolated TNF-R1-DD ligand protein or binding inhibitors (from whatever source derived, including without limitation from recombinant and non-recombinant cell lines) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to TNF-R1-DD ligand protein or binding inhibitor and a carrier) diluents,
30 fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active

ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, G-CSF, Meg-CSF; stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with isolated TNF-R1-DD ligand protein or binding inhibitor, or to minimize side effects caused by the isolated TNF-R1-DD ligand protein or binding inhibitor. Conversely, isolated TNF-R1-DD ligand protein or binding inhibitor may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which isolated TNF-R1-DD ligand protein or binding inhibitor is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of an inflammatory response or condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined

amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of isolated TNF-R1-DD ligand protein or binding inhibitor is administered to a mammal having a condition to be treated. Isolated TNF-R1-DD ligand protein or binding inhibitor may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, isolated TNF-R1-DD ligand protein or binding inhibitor may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering isolated TNF-R1-DD ligand protein or binding inhibitor in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of isolated TNF-R1-DD ligand protein or binding inhibitor used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of isolated TNF-R1-DD ligand protein or binding inhibitor is administered orally, isolated TNF-R1-DD ligand protein or binding inhibitor will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% isolated TNF-R1-DD ligand protein or binding inhibitor, and preferably from about 25 to 90% isolated TNF-R1-DD ligand protein or binding inhibitor. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological

saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of isolated TNF-R1-DD ligand protein or binding inhibitor, and preferably from about 1 to 50% isolated TNF-R1-DD ligand protein or binding inhibitor.

When a therapeutically effective amount of isolated TNF-R1-DD ligand protein or binding inhibitor is administered by intravenous, cutaneous or subcutaneous injection, isolated TNF-R1-DD ligand protein or binding inhibitor will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to isolated TNF-R1-DD ligand protein or binding inhibitor, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of isolated TNF-R1-DD ligand protein or binding inhibitor in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of isolated TNF-R1-DD ligand protein or binding inhibitor with which to treat each individual patient. Initially, the attending physician will administer low doses of isolated TNF-R1-DD ligand protein or binding inhibitor and observe the patient's response. Larger doses of isolated TNF-R1-DD ligand protein or binding inhibitor may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 μ g to about 100 mg of isolated TNF-R1-DD ligand protein or binding inhibitor per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the isolated TNF-R1-DD ligand protein or binding inhibitor will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Isolated TNF-R1-DD ligand protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the TNF-R1-DD ligand protein and which may inhibit TNF-R death domain binding. Such antibodies may be obtained using either the entire TNF-R1-DD ligand protein or fragments of TNF-R1-DD ligand protein as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, *J. Amer. Chem. Soc.* 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, *FEBS Lett.* 211, 10 (1987).

Monoclonal antibodies binding to TNF-R1-DD ligand protein or to complex carbohydrate moieties characteristic of the TNF-R1-DD ligand glycoprotein may be useful diagnostic agents for the immunodetection of TNF-R ligand protein.

Neutralizing monoclonal antibodies binding to TNF-R1-DD ligand protein or to complex carbohydrates characteristic of TNF-R1-DD ligand glycoprotein may also be useful therapeutics for both inflammatory conditions and also in the treatment of some forms of cancer where abnormal expression of TNF-R1-DD ligand protein is involved. These neutralizing monoclonal antibodies are capable of blocking the signaling function of the TNF-R1-DD ligand protein. By blocking the binding of TNF-R1-DD ligand protein, certain biological responses to TNF are either abolished or markedly reduced. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against TNF-R1-DD ligand protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the TNF-R1-DD ligand protein.

Due to the similarity of their sequences to the insulin growth factor binding protein ("IGFBP-5") and fragments thereof which bind to the TNF-R death domain are proteins having TNF-R1-DD ligand protein activity as defined herein. As a result, they are also useful in pharmaceutical compositions, for treating inflammatory conditions and for inhibiting TNF-R death domain binding as described above for TNF-R1-DD ligand proteins generally.

EXAMPLE 1
CLONING OF TNF-R DEATH DOMAIN LIGAND
PROTEIN ENCODING POLYNUCLEOTIDE

A yeast genetic selection method, the "interaction trap" [Gyuris et al, Cell 75:791-803, 1993, which is incorporated herein by reference], was used to screen WI38 cell cDNA libraries (preparation, see below) for proteins that interact with the death domain of the P55 type 1 TNF receptor (TNF-R1-DD). A polynucleotide encoding amino acids 326 to 413 of the P55 type TNF receptor, TNF-R1-DD, was obtained via the polymerase chain reaction (PCR) using a grafting method. This TNF-R1-DD DNA was then cloned into pEG202 by BamHI and SalI sites, generating the bait plasmid, pEG202-TNF-R1-DD. This plasmid contains the HIS3 selectable marker, and expression of the bait, the LexA-TNF-R1-DD fusion protein, is from the strong constitutive ADHI promoter. To create the reporter strain carrying the bait protein, yeast strain EGY48, containing the reporter sequence LexAop-Leu2 in place of the chromosomal LEU2, was transformed with pEG202-TNF-R1-DD and pSH18-34 (Ura+), which carries another reporter sequence. LexAop-lacZ. For screening cDNAs encoding proteins that interact with TNF-R1-DD, the expression vector pJG4-5 (TRP1), containing the WI38 cell cDNA library (see below for the cDNA library construction), was transformed into the above strain (EGY48/pEG202-TNF-R1-DD/pSH18-34) according to the method described by Gietz *et al.*, Nucleic Acids Res., 20:1425 (1992).

cDNA Library Construction:

WI38 cell cDNA library: Double stranded cDNA was prepared from 3ug of WI38 mRNA using reagents provided by the Superscript Choice System

(Gibco/BRL, Gaithersburg, MD) with the following substitutions: the first strand synthesis was primed using an oligo dT/XhoI primer/linker, and the dNTP mix was substituted with a mix containing methyl dCTP (Stratagene, LaJolla, CA). The cDNA was modified at both ends by addition of an EcoRI/NotI/SalI adapter linker and subsequently digested with XhoI. This produced cDNA molecules possessing an EcoRI/NotI/SalI overhang at the 5' end of the gene and an XhoI overhang at the 3' end. These fragments were then ligated into the yeast expression/fusion vector pJG4-5 (Gyuris et al., Cell, 75, 791-803, 1993), which contains at its amino terminus, the influenza virus HA1 epitope tag, the B42 acidic transcription activation domain, and the SV40 nuclear localization signal, all under the control of the galactose-dependent GAL1 promoter. The resulting plasmids were then electroporated into DH10B cells (Gibco/BRL). A total of 7.1×10^6 colonies were plated on LB plates containing 100 ug/ml of ampicillin. These *E. coli* were scraped, pooled, and a large scale plasmid prep was performed using the Wizard Maxi Prep kit (Promega, Madison, WI), yielding 3.2mg of supercoiled plasmid DNA.

WI38 Cell cDNA Screening Results:

1×10^6 transformants were obtained on glucose Ura⁺His⁺Trp⁺ plates. These transformants were pooled and resuspended in a solution of 65% glycerol, 10mM Tris-HCl (pH 7.5), 10 mM MgCl₂ and stored at -80°C in 1mL aliquots. For screening purposes, aliquots of these were diluted 10-fold into Ura⁺His⁺Trp⁺ CM dropout gal/raff medium (containing 2% galactose, 1% raffinose), which induces the expression of the library encoded proteins, and incubated at 30°C for 4 hours. 12×10^6 colony forming units (CFUs) were then plated on standard 10cm galactose X-Gal Ura⁺His⁺Trp⁺Leu⁻ plates at a density of 2×10^5 CFU/plate. After three days at 30°C, about 1,000 colonies were formed (Leu⁻) and of those, sixty-four colonies were LacZ⁻. In order to test if the Leu⁻/LacZ⁻ phenotype was due to the library-encoded protein, the galactose dependency of the phenotype was tested. Expression of the library-encoded proteins was turned off by growth on glucose Ura⁺His⁺Trp⁺ master plates and then retested for galactose-dependency on glucose Ura⁺His⁺Trp⁺Leu⁻, galactose Ura⁺His⁺Trp⁺Leu⁻, glucose X-Gal Ura⁺His⁺Trp⁺, and galactose X-Gal Ura⁺His⁺Trp⁺ plates. Of these, 32 colonies showed galactose-dependent growth on

Leu⁺ plates and galactose-dependent blue color on X-Gal-containing medium (LacZ⁺ phenotype). Total yeast DNA was prepared from these colonies according to the method described previously (Hoffman and Winston, 1987). In order to analyze the cDNA sequences, PCR reactions were performed using the above yeast DNA as a template and oligo primers specific for the vector pJG4-5, flanking the cDNA insertion point. PCR products were purified (Qiagen PCR purification kit), subjected to restriction digest with the enzyme HaeIII, run on 1.8% agarose gels, and the restriction patterns compared. Similar and identical restriction patterns were grouped and representatives of each group were sequenced and compared to Genbank and other databases to identify any sequence homologies.

One clone of unique sequence ("2DD") and three clones with identical sequence ("3DD") were isolated and showed no significant sequence homologies compared to Genbank and other databases. Additionally, four other clones ("20DD") with identical sequence to a portion of human insulin-like growth factor binding protein-5 (Shunichi Shimasaki *et al.*, J. Biol. Chem. 266:10646-10653 (1991)) were isolated. The clones "2DD," "3DD" and "20DD" were chosen for further analysis. Library vector pJG4-5 containing these clones sequences were rescued from yeast by transforming the total yeast DNAs into the *E. coli* strain KC8 and selecting for growth on Trp-ampicillin plates. These putative TNFR1 interacting proteins were then tested further for specificity of interaction with the TNF-R1-DD by the reintroduction of JG4-5 clone into EGY48 derivatives containing a panel of different baits, including bicoid, the cytoplasmic domain of the IL-1 receptor, and TNF-R1-DD. The above clones were found to interact only with the TNF-R1-DD. The interaction between these clones and TNF-R1-DD was thus judged to be specific.

U937 cDNA Screening Results:

A U937 cDNA library was also constructed and screened as described above. 1,020 Leu⁺ colonies were found and of those, 326 colonies were also LacZ⁺. 62 colonies of these Leu⁺/LacZ⁺ colonies showed a galactose-dependent phenotype. One of these clones, 1TU, encodes a novel sequence. Interestingly, two clones, 15TU and 27TU, encode related or identical sequences, except that 27TU contains

about 864 additional nucleotides (or about 288 amino acids) at the 5' end. 15/27TU also encode a novel sequence.

EXAMPLE 2

EXPRESSION OF THE TNF-R1-DD ligand PROTEIN

5 cDNAs encoding TNF-R intracellular ligand proteins were released from the pJG4-5 vector with the appropriate restriction enzymes. For example, EcoRI and XhoI or NotI and XhoI were used to release cDNA from clone 2DD and clone
10 20DD. Where the restriction sites were also present in the internal sequence of the cDNA, PCR was performed to obtain the cDNA. For example, the cDNA fragment encoding "clone 3DD" was obtained through PCR due to the presence of an internal XhoI site. These cDNAs were then cloned into various expression vectors. These included pGEX (Pharmacia) or pMAL (New England Biolabs) for expression as a
15 GST (Glutathione-S-transferase) or MBP (maltose binding protein) fusion protein in E. coli, a pED-based vector for mammalian expression, and pVL or pBlueBacHis (Invitrogen) for baculovirus/insect expression. For the immunodetection of TNF-R intracellular ligand expression in mammalian cells, an epitope sequence, "Flag," was inserted into the translational start site of the pED vector, generating the pED-Flag
20 vector. cDNAs were then inserted into the pED-Flag vector. Thus, the expression of cDNA from pED-Flag yields a protein with an amino terminal Met, followed by the "Flag" sequence. Asp-Tyr-Lys-Asp-Asp-Asp-Lys. Standard DEAE-Dextran or lipofectamine methods were used to transfect COS or CHO dukx cells. Immunodetection of Flag-tagged proteins was achieved using the M2 antibody
25 (Kodak). Moreover, an immunoaffinity column using the M2 antibody, followed by elution with the "Flag" peptide, can be used for the rapid purification of the flag-tagged protein. Similarly, affinity purification of GST-, MBP- or His-tagged fusion proteins can be performed using glutathione, amylose, or nickel columns. Detailed purification protocols are provided by the manufacturers. For many fusion
30 proteins, the TNF-R intracellular ligand can be released by the action of thrombin, factor Xa, or enterokinase cleavage. In the case where highly purified material is required, standard purification procedures, such as ion-exchange, hydrophobic, and

gel filtration chromatography will be applied in addition to the affinity purification step.

Figs. 1 and 2 depict autoradiographs demonstrating the expression of TNF-R1-DD ligand proteins in yeast and mammalian cells. Fig. 1 shows the results of expression of isolated clones of the present invention in yeast. EGY48 was transformed with pJG4-5 containing clone 2DD, 3DD or 20DD. Cells were then grown overnight in the galactose/raffinose medium. Cell lysates were prepared and subject to 4-20% SDS gel electrophoresis, followed by Western blot analysis using anti-HA antibody (12CA5, Boehringer Mannheim, Indianapolis, IN). Fig. 2 shows the results of expression of Flag-2DD and Flag-20DD in COS cells. COS cells were transfected with either pED-Flag (Vector control), Flag-2DD or Flag-20DD plasmid by the lipofectamine method. Thirty µg of each cell lysate were prepared and subjected to 4-20% SDS gel electrophoresis, followed by Western blot analysis using M2 antibody (Kodak). The bands in the Flag-2DD and Flag-20DD lanes indicate significant expression of the respective TNF-R1-DD ligand proteins.

EXAMPLE 3

ASSAYS OF TNF-R DEATH DOMAIN BINDING

Two different methods were used to assay for TNF-R1-DD ligand protein activity. The first assay measures binding in the yeast strain in "interaction trap." the system used here to screen for TNF-R1-DD interacting proteins. In this system, the expression of reporter genes from both LexAop-Leu2 and LexAop-LacZ relies on the interaction between the bait protein, in this case TNF-R1DD, and the prey, the TNF-R intracellular ligand. Thus, one can measure the strength of the interaction by the level of Leu2 or LacZ expression. The most simple method is to measure the activity of the LacZ encoded protein, β-galactosidase. This activity can be judged by the degree of blueness on the X-Gal containing medium or filter. For the quantitative measurement of β-galactosidase activity, standard assays can be found in "Methods in Yeast Genetics" Cold Spring Harbor, New York, 1990 (by Rose, M.D., Winston, F., and Hieter, P.).

The second assay for measuring binding is a cell-free system. An example of a typical assay is described below. Purified GST-TNF-R1-DD fusion

protein (2 ug) was mixed with amylose resins bound with a GST-TNF-R1-DD intracellular ligand for 2 hour at 4°C. The mixture was then centrifuged to separate bound (remained with the beads) and unbound (remained in the supernatant) GST-TNF-R1-DD. After extensive washing, the bound GST-TNF-R1-DD was eluted with maltose and detected by Western blot analysis using a GST antibody. The TNF-R1-DD or the intracellular ligand can also be immobilized on other solid supports, such as on plates or fluorobeads. The binding can then be measured using ELISA or SPA (scintillation proximity assay).

EXAMPLE 4 **CHARACTERIZATION OF TNF-R** **DEATH DOMAIN LIGAND PROTEIN**

Mapping the interaction site in TNF-R1

Many of the key amino acids for TNF-R signaling have been determined by site-directed mutagenesis (Tataglia *et al.*, Cell 74:845-853 (1993). These amino acids are conserved between TNF-R and the Fas antigen, which is required for mediating cytotoxicity and other cellular responses. In order to test if the TNF-R intracellular proteins interact with these residues, the following mutations were constructed: F345A (substitution of phe at amino acid 345 to Ala), R347A, L351A, F345A/R347A/L351A, E369A, W378A and I408A. The ability of the mutant protein to interact with the intracellular ligand in the "interaction trap" system was tested.

Effect on the TNF-mediated response

The effect of the TNF-R intracellular ligands on the TNF-mediated response can be evaluated in cells overexpressing the ligands. A number of TNF-mediated responses, including transient or prolonged responses, can be measured. For example, TNF-induced kinase activity toward either MBP (myelin basic protein) or the N-terminus (amino acids 1-79) of c-jun can be measured in COS cells or CHO cells either transiently or stably overexpressing clone 2DD, 3DD or clone 20DD. The significance of these ligand proteins in TNF-mediated cytotoxicity and other cellular responses can be measured in L929 or U937 overexpressing cells.

Alternatively, other functional assays, such as the induction of gene expression or PGE₂ production after prolonged incubation with TNF, can also be used to measure the TNF mediated response. Conversely, the significance of the TNF-R1-DD ligand proteins in TNF signaling can be established by lowering or eliminating the expression of the ligands. These experiments can be performed using antisense expression or transgenic mice.

Enzymatic or functional assays

The signal transduction events initiated by TNF binding to its receptor are still largely unknown. However, one major result of TNF binding is the stimulation of cellular serine/threonine kinase activity. In addition, TNF has been shown to stimulate the activity of PC-PLC, PLA₂, and sphingomyelinase. Therefore, some of the TNF-R1-DD ligand proteins may possess intrinsic enzymatic activity that is responsible for these activities. Therefore, enzymatic assays can be performed to test this possibility, particularly with those clones that encode proteins with sequence homology to known enzymes. In addition to enzymatic activity, based on the sequence homology to proteins with known function, other functional assays can also be measured.

20

EXAMPLE 5 **ISOLATION OF FULL LENGTH CLONES**

In many cases, cDNAs obtained from the interaction trap method each encode only a portion of the full length protein. For example, based on identity and sequence and the lack of the initiating methionine codon, clones 2DD, 3DD and 20DD apparently do not encode full length proteins. Therefore, it is desirable to isolate full length clones. The cDNAs obtained from the screening, such as clone 2DD, are used as probes, and the cDNA libraries described herein, or alternatively phage cDNA libraries, are screened to obtain full length clones in accordance with known methods (see for example, "Molecular Cloning, A Laboratory Manual", by Sambrook et al., 1989 Cold Spring Harbor).

30

EXAMPLE 6
ANTIBODIES SPECIFIC FOR TNF-R
INTRACELLULAR LIGAND PROTEIN

5 Antibodies specific for TNF-R intracellular ligand proteins can be produced using purified recombinant protein, as described in Example 2, as antigen. Both polyclonal and monoclonal antibodies will be produced using standard techniques, such as those described in "Antibodies, a Laboratory Manual" by Ed Harlow and David Lane (1988), Cold Spring Harbor Laboratory.

10

EXAMPLE 7
CHARACTERIZATION OF
CLONES 1TU AND 15/27TU

15 **Specificity of Interaction**

 The specificity of clones 1TU, 15TU and 27TU was tested using a panel of baits. The ability of these clones to bind the TNF-R death domain was compared to their binding to the intracellular domain of the second TNF-R (TNF-R p75_{IC}), the entire intracellular domain of TNF-R (TNF-R p55_{IC}), the death domain of the fas antigen (which shares 28% identity with TNF-R-DD) (Fas_{DD}), the *Drosophila* transcription factor bicoid, and a region of the IL-1 receptor known to be critical for signalling (IL-1R₄₇₇₋₅₂₇). As shown in Table 1, none of these clones interacted with TNF-R p75_{IC} or Fas_{DD}, and only 1TU interacted with bicoid. In contrast, both 1TU and 15TU bound the cytoplasmic domain of the p55 TNF-R, as well as residues 477-25 527 of the IL-1R. 27TU interacted relatively weakly with these sequences.

Table 1

clone	TNF-R _{DD}	TNF-R p75 _{IC}	TNF-R p55 _{IC}	Fas _{DD}	bicoid	IL-1R (477-527)
1TU	+++	-	+++	-	++	+++
15TU	+++	±	+++	-	-	++
27TU	+++	-	+	-	-	±

30

Interaction with Amino Acids Critical for Signalling

The ability of each clone to interact with four single-site mutations in the TNF-R death domain (each known to abolish signalling) was measured. As shown in Table 2, each of the clones interacted less strongly with the death domain mutants than with the wild type death domain, suggesting that these clones may bind critical residues *in vivo*.

Table 2

clone	TNF-R _{DD}	F345A	L351A	W378A	I408A
1TU	+++	+	++	++	+
15TU	+++	+	+	++	++
27TU	+++	+	+	±	++

Expression of 1TU, 15TU and 27TU

Fig. 3 depicts an autoradiograph demonstrating the expression of clones 1TU, 15TU and 27TU in yeast (A) and COS cells (B).

In (A): EGY48 was transformed with pJG4-5 containing clones 1TU, 15TU or 27TU. Cells were then grown overnight in galactose/raffinose medium. Cell lysates were prepared and subjected to 4-20% SDS gel electrophoresis, followed by Western blot analysis using anti-HA antibody (12CA5, Boehringer Mannheim).

In (B): COS cells were transfected with pED-Flag containing clones 1TU, 15TU and 27TU. Cell lysates were prepared and analyzed by Western blot using anti-Flag antibody (M2, Kodak).

Specific Binding of 1TU and 27TU to TNF-R1-DD

The interaction of 1TU and 27TU with TNF-R1-DD was tested using purified bacterially expressed fusion proteins. As shown in Fig. 4, MBP fusion proteins containing 1TU or 27TU bound only to TNF-R1-DD expressed as a GST fusion protein, but not to GST protein alone. In the control experiment, MBP protein did not bind either GST or GST/TNF-R1-DD. These results indicate that

1TU and 27TU bound specifically to the TNF-R1 death domain *in vitro*, confirming the data obtained in the interaction trap.

15TU and 27TU Activation of JNK Activity

5 The jun N-terminal kinase (JNK) is normally activated within 15 min of TNF treatment in COS cells. 15TU and 27TU were cotransfected with an epitope tagged version of JNK, HA-JNK, in duplicate. After TNF treatment, JNK was immunoprecipitated with anti-HA antibody and JNK activity was measured in immunoprecipitation kinase assays, using GST-c-jun (amino acids 1-79) as
10 substrate). Reactions were electrophoresed on SDS-PAGE. As shown in Fig. 5, transfection of 15TU and 27TU, but not vector alone, into COS cells activated JNK even in the absence of TNF, suggesting that these clones are involved in signal transduction of TNF and the pathway leading to JNK activation *in vivo*.

15

EXAMPLE 8 ISOLATION, EXPRESSION AND ASSAY OF CLONE 3TW

 Clone 3TW was isolated from the WI38 cDNA library using clone 3DD as
20 a probe. Clone 3TW was expressed. Fig. 6 is an autoradiograph which demonstrates expression of 3TW (indicated by arrow).

 An antisense oligonucleotide was derived from the sequence of clone 3TW. The antisense oligonucleotide was assayed to determine its ability to inhibit TNF-induced cPLA₂ phosphorylation. Fig. 7 depicts the results of that experiment.
25 Activity of the antisense oligonucleotide (3TWAS) was compared with the full-length clone (3TWFL), Flag-3TW full length (3TWFLflag) and pED-flag vector (pEDflag). The antisense oligonucleotide inhibited phosphorylation.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lin, Lih-Ling
Chen, Jennifer H.
Schievella, Andrea
Graham, James

(ii) TITLE OF INVENTION: NOVEL TNF RECEPTOR DEATH DOMAIN LIGAND
PROTEINS AND INHIBITORS OF LIGAND BINDING

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Genetics Institute, Inc.
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02140

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Brown, Scott A.
(B) REGISTRATION NUMBER: 32,724
(C) REFERENCE/DOCKET NUMBER: GI5232B

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 498-8224
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2158 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 2..1231

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

C AGC AAT GCA GGT GAT GGA CCA GGT GGC GAG GGC AGT GTT CAC CTG
Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu Gly Ser Val His Leu

45

1	5	10	15	
GCA AGC TCT CGG GGC ACT TTG TCT GAT AGT GAA ATT GAG ACC AAC TCT Ala Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu Ile Glu Thr Asn Ser	20	25	30	94
GCC ACA AGC ACC ATC TTT GGT AAA GCC CAC AGC TTG AAG CCA AGC ATA Ala Thr Ser Thr Ile Phe Gly Lys Ala His Ser Leu Lys Pro Ser Ile	35	40	45	142
AAG GAG AAG CTG GCA GGC AGC CCC ATT CGT ACT TCT GAA GAT GTG AGC Lys Glu Lys Leu Ala Gly Ser Pro Ile Arg Thr Ser Glu Asp Val Ser	50	55	60	190
CAG CGA GTC TAT CTC TAT GAG GGA CTC CTA GGC AAA GAG CGT TCT ACT Gln Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly Lys Glu Arg Ser Thr	65	70	75	238
TTA TGG GAC CAA ATG CAA TTC TGG GAA GAT GCC TTC TTA GAT GCT GTG Leu Trp Asp Gln Met Gln Phe Trp Glu Asp Ala Phe Leu Asp Ala Val	80	85	90	286
ATG TTG GAG AGA GAA GGG ATG GGT ATG GAC CAG GGT CCC CAG GAA ATG Met Leu Glu Arg Glu Gly Met Gly Met Asp Gln Gly Pro Gln Glu Met	100	105	110	334
ATC GAC AGG TAC CTG TCC CTT GGA GAA CAT GAC CGG AAG CGC CTG GAA Ile Asp Arg Tyr Leu Ser Leu Gly Glu His Asp Arg Lys Arg Leu Glu	115	120	125	382
GAT GAT GAA GAT CGC TTG CTG GCC ACA CTT CTG CAC AAC CTC ATC TCC Asp Asp Glu Asp Arg Leu Leu Ala Thr Leu Leu His Asn Leu Ile Ser	130	135	140	430
TAC ATG CTG CTG ATG AAG GTA AAT AAG AAT GAC ATC CGC AAG AAG GTG Tyr Met Leu Leu Met Lys Val Asn Lys Asn Asp Ile Arg Lys Lys Val	145	150	155	478
AGG CGC CTA ATG GGA AAG TCG CAC ATT GGG CTT GTG TAC AGC CAG CAA Arg Arg Leu Met Gly Lys Ser His Ile Gly Leu Val Tyr Ser Gln Gln	160	165	170	526
ATC AAT GAG GTG CTT GAT CAG CTG GCG AAC CTG AAT GGA CGC GAT CTC Ile Asn Glu Val Leu Asp Gln Leu Ala Asn Leu Asn Gly Arg Asp Leu	180	185	190	574
TCT ATC TGG TCC AGT GGC AGC CGG CAC ATG AAG AAG CAG ACA TTT GTG Ser Ile Trp Ser Ser Gly Ser Arg His Met Lys Lys Gln Thr Phe Val	195	200	205	622
GTA CAT GCA GGG ACA GAT ACA AAC GGA GAT ATC TTT TTC ATG GAG GTG Val His Ala Gly Thr Asp Thr Asn Gly Asp Ile Phe Phe Met Glu Val	210	215	220	670
TGC GAT GAC TGT GTG GTG TTG CGT AGT AAC ATC GGA ACA GTG TAT GAG Cys Asp Asp Cys Val Val Leu Arg Ser Asn Ile Gly Thr Val Tyr Glu	225	230	235	718
CGC TGG TGG TAC GAG AAG CTC ATC AAC ATG ACC TAC TGT CCC AAG ACG Arg Trp Trp Tyr Glu Lys Leu Ile Asn Met Thr Tyr Cys Pro Lys Thr	240	245	250	766
AAG GTG TTG TGC TTG TGG CGT AGA AAT GGC TCT GAG ACC CAG CTC AAC Lys Val Leu Cys Leu Trp Arg Arg Asn Gly Ser Glu Thr Gln Leu Asn	260	265	270	814

AAG TTC TAT ACT AAA AAG TGT CGG GAG CTG TAC TAC TGT GTG AAG GAC	862
Lys Phe Tyr Thr Lys Lys Cys Arg Glu Leu Tyr Tyr Cys Val Lys Asp	
275 280 285	
AGC ATG GAG CGC GCT GCC GCC CGA CAG CAA AGC ATC AAA CCC GGA CCT	910
Ser Met Glu Arg Ala Ala Ala Arg Gln Gln Ser Ile Lys Pro Gly Pro	
290 295 300	
GAA TTG GGT GGC GAG TTC CCT GTG CAG GAC CTG AAG ACT GGT GAG GGT	958
Glu Leu Gly Gly Glu Phe Pro Val Gln Asp Leu Lys Thr Gly Glu Gly	
305 310 315	
GGC CTG CTG CAG GTG ACC CTG GAA GGG ATC AAC CTC AAA TTC ATG CAC	1006
Gly Leu Leu Gln Val Thr Leu Glu Gly Ile Asn Leu Lys Phe Met His	
320 325 330 335	
AAT CAG GTT TTC ATA GAG CTG AAT CAC ATT AAA AAG TGC AAT ACA GTT	1054
Asn Gln Val Phe Ile Glu Leu Asn His Ile Lys Lys Cys Asn Thr Val	
340 345 350	
CGA GGC GTC TTT GTC CTG GAG GAA TTT GTT CCT GAA ATT AAA GAA GTG	1102
Arg Gly Val Phe Val Leu Glu Glu Phe Val Pro Glu Ile Lys Glu Val	
355 360 365	
GTG AGC CAC AAG TAC AAG ACA CCA ATG GCC CAC GAA ATC TGC TAC TCC	1150
Val Ser His Lys Tyr Lys Thr Pro Met Ala His Glu Ile Cys Tyr Ser	
370 375 380	
GTA TTA TGT CTC TTC TCG TAC GTG GCT GCA GTT CAT AGC AGT GAG GAA	1198
Val Leu Cys Leu Phe Ser Tyr Val Ala Ala Val His Ser Ser Glu Glu	
385 390 395	
GAT CTC AGA ACC CCG CCC CGG CCT GTC TCT AGC TGATGGAGAG GGGCTACGCA	1251
Asp Leu Arg Thr Pro Pro Arg Pro Val Ser Ser	
400 405 410	
GCTGCCCCAG CCCAGGGCAC GCCCCTGGCC CCTTGCTGTT CCCAAGTGCA CGATGCTGCT	1311
GTGACTGAGG AGTGGATGAT GCTCGTGTGT CCTCTGCAAG CCCCTGCTG TGGCTTGGGT	1371
GGGTACCGGT TATGTGTCCC TCTGAGTGTG TCTTGAGCGT GTCCACCTTC TCCCTCTCCA	1431
CTCCCAGAAG ACCAAACTGC CTTCCCCTCA GGGCTCAAGA ATGTGTACAG TCTGTGGGGC	1491
CGGTGTGAAC CCACTATTTT GTGTCCTTGA GACATTTGTG TTGTGGTTCC TTGTCTTGT	1551
CCCTGGCGTT AACTGTCCAC TGCAAGAGTC TGGCTCTCCC TTCTCTGTGA CCCGGCATGA	1611
CTGGGCGCCT GGAGCAGTTT CACTCTGTGA GGAGTGAGGG AACCCCTGGGG CTCACCCTCT	1671
CAGAGGAAGG GCACAGAGAG GAAGGGAAGA ATTGGGGGGC AGCCGGAGTG AGTGGCAGCC	1731
TCCCTGCTTC CTTCTGCATT CCCAAGCCGG CAGCTACTGC CCAGGGCCCG CAGTGTTGGC	1791
TGCTGCCTGC CACAGCCTCT GTGACTGCAG TGGAGCGGCG AATCCCTGT GGCCTGCCAC	1851
GCCTTCGGCA TCAGAGGATG GAGTGGTCGA GGCTAGTGGA GTCCCAGGGA CCGCTGGCTG	1911
CTCTGCCTGA GCATCAGGGA GGGGGCAGGA AAGACCAAGC TGGGTTTGCA CATCTGTCTG	1971
CAGGCTGTCT CTCCAGGCAC GGGGTGTCAG GAGGGAGAGA CAGCCTGGGT ATGGGCAAGA	2031
AATGACTGTA AATATTTTCAG CCCACATTA TTTATAGAAA ATGTACAGTT GTGTGAATGT	2091
GAAATAAATG TCCTCACCTC CCAAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	2151
AAAAAA	2158

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 410 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu Gly Ser Val His Leu Ala
 1 5 10 15
 Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu Ile Glu Thr Asn Ser Ala
 20 25 30
 Thr Ser Thr Ile Phe Gly Lys Ala His Ser Leu Lys Pro Ser Ile Lys
 35 40 45
 Glu Lys Leu Ala Gly Ser Pro Ile Arg Thr Ser Glu Asp Val Ser Gln
 50 55 60
 Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly Lys Glu Arg Ser Thr Leu
 65 70 75 80
 Trp Asp Gln Met Gln Phe Trp Glu Asp Ala Phe Leu Asp Ala Val Met
 85 90 95
 Leu Glu Arg Glu Gly Met Gly Met Asp Gln Gly Pro Gln Glu Met Ile
 100 105 110
 Asp Arg Tyr Leu Ser Leu Gly Glu His Asp Arg Lys Arg Leu Glu Asp
 115 120 125
 Asp Glu Asp Arg Leu Leu Ala Thr Leu Leu His Asn Leu Ile Ser Tyr
 130 135 140
 Met Leu Leu Met Lys Val Asn Lys Asn Asp Ile Arg Lys Lys Val Arg
 145 150 155 160
 Arg Leu Met Gly Lys Ser His Ile Gly Leu Val Tyr Ser Gln Gln Ile
 165 170 175
 Asn Glu Val Leu Asp Gln Leu Ala Asn Leu Asn Gly Arg Asp Leu Ser
 180 185 190
 Ile Trp Ser Ser Gly Ser Arg His Met Lys Lys Gln Thr Phe Val Val
 195 200 205
 His Ala Gly Thr Asp Thr Asn Gly Asp Ile Phe Phe Met Glu Val Cys
 210 215 220
 Asp Asp Cys Val Val Leu Arg Ser Asn Ile Gly Thr Val Tyr Glu Arg
 225 230 235 240
 Trp Trp Tyr Glu Lys Leu Ile Asn Met Thr Tyr Cys Pro Lys Thr Lys
 245 250 255
 Val Leu Cys Leu Trp Arg Arg Asn Gly Ser Glu Thr Gln Leu Asn Lys
 260 265 270
 Phe Tyr Thr Lys Lys Cys Arg Glu Leu Tyr Tyr Cys Val Lys Asp Ser
 275 280 285

Met Glu Arg Ala Ala Ala Arg Gln Gln Ser Ile Lys Pro Gly Pro Glu
 290 295 300

Leu Gly Gly Glu Phe Pro Val Gln Asp Leu Lys Thr Gly Glu Gly Gly
 305 310 315 320

Leu Leu Gln Val Thr Leu Glu Gly Ile Asn Leu Lys Phe Met His Asn
 325 330 335

Gln Val Phe Ile Glu Leu Asn His Ile Lys Lys Cys Asn Thr Val Arg
 340 345 350

Gly Val Phe Val Leu Glu Glu Phe Val Pro Glu Ile Lys Glu Val Val
 355 360 365

Ser His Lys Tyr Lys Thr Pro Met Ala His Glu Ile Cys Tyr Ser Val
 370 375 380

Leu Cys Leu Phe Ser Tyr Val Ala Ala Val His Ser Ser Glu Glu Asp
 385 390 395 400

Leu Arg Thr Pro Pro Arg Pro Val Ser Ser
 405 410

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 826 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2..415

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

G GAG GTG CAG GAC CTC TTC GAA GCC CAG GGC AAT GAC CGA CTG AAG	46
Glu Val Gln Asp Leu Phe Glu Ala Gln Gly Asn Asp Arg Leu Lys	
1 5 10 15	
CTG CTG GTG CTG TAC AGT GGA GAG GAT GAT GAG CTG CTA CAG CGG GCA	94
Leu Leu Val Leu Tyr Ser Gly Glu Asp Asp Glu Leu Leu Gln Arg Ala	
20 25 30	
GCT GCC GGG GGC TTG GCC ATG CTT ACC TCC ATG CGG CCC ACG CTC TGC	142
Ala Ala Gly Gly Leu Ala Met Leu Thr Ser Met Arg Pro Thr Leu Cys	
35 40 45	
AGC CGC ATT CCC CAA GTG ACC ACA CAC TGG CTG GAG ATC CTG CAG GCC	190
Ser Arg Ile Pro Gln Val Thr Thr His Trp Leu Glu Ile Leu Gln Ala	
50 55 60	
CTG CTT CTG AGC TCC AAC CAG GAG CTG CAG CAC CGG GGT GCT GTG GTG	238
Leu Leu Leu Ser Ser Asn Gln Glu Leu Gln His Arg Gly Ala Val Val	
65 70 75	
GTG CTG AAC ATG GTG GAG GCC TCG AGG GAG ATT GCC AGC ACC CTG ATG	286
Val Leu Asn Met Val Glu Ala Ser Arg Glu Ile Ala Ser Thr Leu Met	
80 85 90 95	

GAG AGT GAG ATG ATG GAG ATC TTG TCA GTG CTA GCT AAG GGT GAC CAC 334
 Glu Ser Glu Met Met Glu Ile Leu Ser Val Leu Ala Lys Gly Asp His 110
 100 105

AGC CCT GTC ACA AGG GCT GCT GCA GCC TGC CTG GAC AAA GCA GTG GAA 382
 Ser Pro Val Thr Arg Ala Ala Ala Cys Leu Asp Lys Ala Val Glu 125
 115 120

TAT GGG CTT ATC CAA CCC AAC CAA GAT GGA GAG TGAGGGGGTT GTCCCTGGGC 435
 Tyr Gly Leu Ile Gln Pro Asn Gln Asp Gly Glu 135
 130

CCAAGGCTCA TGCACACGCT ACCTATTGTG GCACGGAGAG TAAGGACGGA AGCAGCTTTG 495

GCTGGTGGTG GCTGGCATGC CCAATACTCT TGCCCATCCT CGCTTGCTGC CCTAGGATGT 555

CCTCTGTTCT GAGTCAGCGG CCACGTTTCAG TCACACAGCC CTGCTTGGCC AGCACTGCCT 615

GCAGCCTCAC TCAGAGGGGC CCTTTTTCTG TACTACTGTA GTCAGCTGGG AATGGGGAAG 675

GTGCATCCCA ACACAGCCTG TGGATCCTGG GGCATTTGGA AGGGCGCACA CATCAGCAGC 735

CTCACCAGCT GTGAGCCTGC TATCAGGCCT GCCCCTCCAA TAAAAGTGTG TAGAACTCCA 795

AAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 826

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 138 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Val Gln Asp Leu Phe Glu Ala Gln Gly Asn Asp Arg Leu Lys Leu 15
 1 5 10

Leu Val Leu Tyr Ser Gly Glu Asp Asp Glu Leu Leu Gln Arg Ala Ala 30
 20 25 30

Ala Gly Gly Leu Ala Met Leu Thr Ser Met Arg Pro Thr Leu Cys Ser 45
 35 40 45

Arg Ile Pro Gln Val Thr Thr His Trp Leu Glu Ile Leu Gln Ala Leu 60
 50 55 60

Leu Leu Ser Ser Asn Gln Glu Leu Gln His Arg Gly Ala Val Val Val 80
 65 70 75 80

Leu Asn Met Val Glu Ala Ser Arg Glu Ile Ala Ser Thr Leu Met Glu 95
 85 90 95

Ser Glu Met Met Glu Ile Leu Ser Val Leu Ala Lys Gly Asp His Ser 110
 100 105 110

Pro Val Thr Arg Ala Ala Ala Ala Cys Leu Asp Lys Ala Val Glu Tyr 125
 115 120 125

Gly Leu Ile Gln Pro Asn Gln Asp Gly Glu 135
 130

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 722 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2..559

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

G GAG AAG CCG CTG CAC GCC CTG CTG CAC GGC CGC GGG GTT TGC CTC	46
Glu Lys Pro Leu His Ala Leu Leu His Gly Arg Gly Val Cys Leu	
1 5 10 15	
AAC GAA AAG AGC TAC CGC GAG CAA GTC AAG ATC GAG AGA GAC TCC CGT	94
Asn Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile Glu Arg Asp Ser Arg	
20 25 30	
GAG CAC GAG GAG CCC ACC ACC TCT GAG ATG GCC GAG GAG ACC TAC TCC	142
Glu His Glu Glu Pro Thr Thr Ser Glu Met Ala Glu Glu Thr Tyr Ser	
35 40 45	
CCC AAG ATC TTC CGG CCC AAA CAC ACC CGC ATC TCC GAG CTG AAG GCT	190
Pro Lys Ile Phe Arg Pro Lys His Thr Arg Ile Ser Glu Leu Lys Ala	
50 55 60	
GAA GCA GTG AAG AAG GAC CGC AGA AAG AAG CTG ACC CAG TCC AAG TTT	238
Glu Ala Val Lys Lys Asp Arg Arg Lys Lys Leu Thr Gln Ser Lys Phe	
65 70 75	
GTC GGG GGA GCC GAG AAC ACT GCC CAC CCC CGG ATC ATC TCT GAA CCT	286
Val Gly Gly Ala Glu Asn Thr Ala His Pro Arg Ile Ile Ser Glu Pro	
80 85 90 95	
GAG ATG AGA CAG GAG TCT GAG CAG GGC CCC TGC CGC AGA CAC ATG GAG	334
Glu Met Arg Gln Glu Ser Glu Gln Gly Pro Cys Arg Arg His Met Glu	
100 105 110	
GCT TCC CTG CAG GAG CTC AAA GCC AGC CCA CGC ATG GTG CCC CGT GCT	382
Ala Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg Met Val Pro Arg Ala	
115 120 125	
GTG TAC CTG CCC AAT TGT GAC CGC AAA GGA TTC TAC AAG AGA AAG CAG	430
Val Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe Tyr Lys Arg Lys Gln	
130 135 140	
TGC AAA CCT TCC CGT GGC CGC AAG CGT GGC ATC TGC TGG TGC GTG GAC	478
Cys Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile Cys Trp Cys Val Asp	
145 150 155	
AAG TAC GGG ATG AAG CTG CCA GGC ATG GAG TAC GTT GAC GGG GAC TTT	526
Lys Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr Val Asp Gly Asp Phe	
160 165 170 175	
CAG TGC CAC ACC TTC GAC AGC AGC AAC GTT GAG TGATGCGTCC CCCCCAACC	579
Gln Cys His Thr Phe Asp Ser Ser Asn Val Glu	
180 185	
TTTCCCTCAC CCCCTTCCAC CCCAGCCCC GACTCCAGCC AGCGCCTCCC TCCACCCACG	639

GACGCCACTC ATTTCATCTC ATTTAAGGGA AAAATATATA TCTATCTATT TGAGGAAAAA
 AAAAAAAAAA AAAAAAAAAA AAA

699

722

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 186 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Lys Pro Leu His Ala Leu Leu His Gly Arg Gly Val Cys Leu Asn
 1 5 10 15
 Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile Glu Arg Asp Ser Arg Glu
 20 25 30
 His Glu Glu Pro Thr Thr Ser Glu Met Ala Glu Glu Thr Tyr Ser Pro
 35 40 45
 Lys Ile Phe Arg Pro Lys His Thr Arg Ile Ser Glu Leu Lys Ala Glu
 50 55 60
 Ala Val Lys Lys Asp Arg Arg Lys Lys Leu Thr Gln Ser Lys Phe Val
 65 70 75 80
 Gly Gly Ala Glu Asn Thr Ala His Pro Arg Ile Ile Ser Glu Pro Glu
 85 90 95
 Met Arg Gln Glu Ser Glu Gln Gly Pro Cys Arg Arg His Met Glu Ala
 100 105 110
 Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg Met Val Pro Arg Ala Val
 115 120 125
 Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe Tyr Lys Arg Lys Gln Cys
 130 135 140
 Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile Cys Trp Cys Val Asp Lys
 145 150 155 160
 Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr Val Asp Gly Asp Phe Gln
 165 170 175
 Cys His Thr Phe Asp Ser Ser Asn Val Glu
 180 185

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1023 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS

(B) LOCATION: 57..875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCCTGCACTC TCGCTCTCCT GCCCCACCCC GAGGTAAAGG GGGCGACTAA GAGAAG	56
ATG GTG TTG CTC ACC GCG GTC CTC CTG CTG CTG GCC GCC TAT GCG GGG Met Val Leu Leu Thr Ala Val Leu Leu Leu Leu Ala Ala Tyr Ala Gly	104
1 5 10 15	
CCG GCC CAG AGC CTG GGC TCC TTC GTG CAC TGC GAG CCC TGC GAC GAG Pro Ala Gln Ser Leu Gly Ser Phe Val His Cys Glu Pro Cys Asp Glu	152
20 25 30	
AAA GCC CTC TCC ATG TGC CCC CCC AGC CCC CTG GGC TGC GAG CTG GTC Lys Ala Leu Ser Met Cys Pro Pro Ser Pro Leu Gly Cys Glu Leu Val	200
35 40 45	
AAG GAG CCG GGC TGC GGC TGC TGC ATG ACC TGC GCC CTG GCC GAG GGG Lys Glu Pro Gly Cys Gly Cys Cys Met Thr Cys Ala Leu Ala Glu Gly	248
50 55 60	
CAG TCG TGC GGC GTC TAC ACC GAG CGC TGC GCC CAG GGG CTG CGC TGC Gln Ser Cys Gly Val Tyr Thr Glu Arg Cys Ala Gln Gly Leu Arg Cys	296
65 70 75 80	
CTC CCC CGG CAG GAC GAG GAG AAG CCG CTG CAC GCC CTG CTG CAC GGC Leu Pro Arg Gln Asp Glu Glu Lys Pro Leu His Ala Leu Leu His Gly	344
85 90 95	
CGC GGG GTT TGC CTC AAC GAA AAG AGC TAC CGC GAG CAA GTC AAG ATC Arg Gly Val Cys Leu Asn Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile	392
100 105 110	
GAG AGA GAC TCC CGT GAG CAC GAG GAG CCC ACC ACC TCT GAG ATG GCC Glu Arg Asp Ser Arg Glu His Glu Glu Pro Thr Thr Ser Glu Met Ala	440
115 120 125	
GAG GAG ACC TAC TCC CCC AAG ATC TTC CGG CCC AAA CAC ACC CGC ATC Glu Glu Thr Tyr Ser Pro Lys Ile Phe Arg Pro Lys His Thr Arg Ile	488
130 135 140	
TCC GAG CTG AAG GCT GAA GCA GTG AAG AAG GAC CGC AGA AAG AAG CTG Ser Glu Leu Lys Ala Glu Ala Val Lys Lys Asp Arg Arg Lys Lys Leu	536
145 150 155 160	
ACC CAG TCC AAG TTT GTC GGG GGA GCC GAG AAC ACT GCC CAC CCC CGG Thr Gln Ser Lys Phe Val Gly Gly Ala Glu Asn Thr Ala His Pro Arg	584
165 170 175	
ATC ATC TCT GCA CCT GAG ATG AGA CAG GAG TCT GAG CAG GGC CCC TGC Ile Ile Ser Ala Pro Glu Met Arg Gln Glu Ser Glu Gln Gly Pro Cys	632
180 185 190	
CGC AGA CAC ATG GAG GCT TCC CTG CAG GAG CTC AAA GCC AGC CCA CGC Arg Arg His Met Glu Ala Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg	680
195 200 205	
ATG GTG CCC CGT GCT GTG TAC CTG CCC AAT TGT GAC CGC AAA GGA TTC Met Val Pro Arg Ala Val Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe	728
210 215 220	
TAC AAG AGA AAG CAG TGC AAA CCT TCC CGT GGC CGC AAG CGT GGC ATC Tyr Lys Arg Lys Gln Cys Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile	776
225 230 235 240	

TGC TGG TGC GTG GAC AAG TAC GGG ATG AAG CTG CCA GGC ATG GAG TAC 824
 Cys Trp Cys Val Asp Lys Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr 255
 245 250
 GTT GAC GGG GAC TTT CAG TGC CAC ACC TTC GAC AGC AGC AAC GTT GAG 872
 Val Asp Gly Asp Phe Gln Cys His Thr Phe Asp Ser Ser Asn Val Glu 270
 260 265
 TGATGCGTCC CCCCCCAACC TTTCCTCACC CCCCTCCCAC CCCAGCCCC GACTCCAGCC 932
 AGCGCCTCCC TCCACCCCAG GACGCCACTC ATTCATCTC ATTTAAGGGA AAAATATATA 992
 TCTATCTATT TGAAAAAAAA AAAAAAACC C 1023

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 272 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Val Leu Leu Thr Ala Val Leu Leu Leu Leu Ala Ala Tyr Ala Gly
 1 5 10 15
 Pro Ala Gln Ser Leu Gly Ser Phe Val His Cys Glu Pro Cys Asp Glu
 20 25 30
 Lys Ala Leu Ser Met Cys Pro Pro Ser Pro Leu Gly Cys Glu Leu Val
 35 40 45
 Lys Glu Pro Gly Cys Gly Cys Cys Met Thr Cys Ala Leu Ala Glu Gly
 50 55 60
 Gln Ser Cys Gly Val Tyr Thr Glu Arg Cys Ala Gln Gly Leu Arg Cys
 65 70 75 80
 Leu Pro Arg Gln Asp Glu Glu Lys Pro Leu His Ala Leu Leu His Gly
 85 90 95
 Arg Gly Val Cys Leu Asn Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile
 100 105 110
 Glu Arg Asp Ser Arg Glu His Glu Glu Pro Thr Thr Ser Glu Met Ala
 115 120 125
 Glu Glu Thr Tyr Ser Pro Lys Ile Phe Arg Pro Lys His Thr Arg Ile
 130 135 140
 Ser Glu Leu Lys Ala Glu Ala Val Lys Lys Asp Arg Arg Lys Lys Leu
 145 150 155 160
 Thr Gln Ser Lys Phe Val Gly Gly Ala Glu Asn Thr Ala His Pro Arg
 165 170 175
 Ile Ile Ser Ala Pro Glu Met Arg Gln Glu Ser Glu Gln Gly Pro Cys
 180 185 190
 Arg Arg His Met Glu Ala Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg
 195 200 205
 Met Val Pro Arg Ala Val Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe
 210 215 220

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Tyr Lys Arg Lys Gln Cys Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile
225                230                235                240

Cys Trp Cys Val Asp Lys Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr
                245                250                255

Val Asp Gly Asp Phe Gln Cys His Thr Phe Asp Ser Ser Asn Val Glu
                260                265                270

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1694 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..931

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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C TCT CTC AAG GCC AAC ATC CCT GAG GTG GAA GCT GTC CTC AAC ACC      46
Ser Leu Lys Ala Asn Ile Pro Glu Val Glu Ala Val Leu Asn Thr
  1                5                10                15

GAC AGG AGT TTG GTG TGT GAT GGG AAG AGG GGC TTA TTA ACT CGT CTG      94
Asp Arg Ser Leu Val Cys Asp Gly Lys Arg Gly Leu Leu Thr Arg Leu
                20                25                30

CTG CAG GTC ATG AAG AAG GAG CCA GCA GAG TCG TCT TTC AGG TTT TGG      142
Leu Gln Val Met Lys Lys Glu Pro Ala Glu Ser Ser Phe Arg Phe Trp
                35                40                45

CAA GCT CGG GCT GTG GAG AGT TTC CTC CGA GGG ACC ACC TCC TAT GCA      190
Gln Ala Arg Ala Val Glu Ser Phe Leu Arg Gly Thr Thr Ser Tyr Ala
                50                55                60

GAC CAG ATG TTC CTG CTG AAG CGA GGC CTC TTG GAG CAC ATC CTT TAC      238
Asp Gln Met Phe Leu Leu Lys Arg Gly Leu Leu Glu His Ile Leu Tyr
                65                70                75

TGC ATT GTG GAC AGC GAG TGT AAG TCA AGG GAT GTG CTC CAG AGT TAC      286
Cys Ile Val Asp Ser Glu Cys Lys Ser Arg Asp Val Leu Gln Ser Tyr
                80                85                90                95

TTT GAC CTC CTG GGG GAG CTG ATG AAG TTC AAC GTT GAT GCA TTC AAG      334
Phe Asp Leu Leu Gly Glu Leu Met Lys Phe Asn Val Asp Ala Phe Lys
                100                105                110

AGA TTC AAT AAA TAT ATC AAC ACC GAT GCA AAG TTC CAG GTA TTC CTG      382
Arg Phe Asn Lys Tyr Ile Asn Thr Asp Ala Lys Phe Gln Val Phe Leu
                115                120                125

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AAG CAG ATC AAC AGC TCC CTG GTG GAC TCC AAC ATG CTG GTG CGC TGT Lys Gln Ile Asn Ser Ser Leu Val Asp Ser Asn Met Leu Val Arg Cys 130 135 140	430
GTC ACT CTG TCC CTG GAC CGA TTT GAA AAC CAG GTG GAT ATG AAA GTT Val Thr Leu Ser Leu Asp Arg Phe Glu Asn Gln Val Asp Met Lys Val 145 150 155	478
GCC GAG GTA CTG TCT GAA TGC CGC CTG CTC GCC TAC ATA TCC CAG GTG Ala Glu Val Leu Ser Glu Cys Arg Leu Leu Ala Tyr Ile Ser Gln Val 160 165 170 175	526
CCC ACG CAG ATG TCC TTC CTC TTC CGC CTC ATC AAC ATC ATC CAC GTG Pro Thr Gln Met Ser Phe Leu Phe Arg Leu Ile Asn Ile Ile His Val 180 185 190	574
CAG ACG CTG ACC CAG GAG AAC GTC AGC TGC CTC AAC ACC AGC CTG GTG Gln Thr Leu Thr Gln Glu Asn Val Ser Cys Leu Asn Thr Ser Leu Val 195 200 205	622
ATC CTG ATG CTG GCC CGA CGG AAA GAG CGG CTG CCC CTG TAC CTG CGG Ile Leu Met Leu Ala Arg Arg Lys Glu Arg Leu Pro Leu Tyr Leu Arg 210 215 220	670
CTG CTG CAG CGG ATG GAG CAC AGC AAG AAG TAC CCC GGC TTC CTG CTC Leu Leu Gln Arg Met Glu His Ser Lys Lys Tyr Pro Gly Phe Leu Leu 225 230 235	718
AAC AAC TTC CAC AAC CTG CTG CGC TTC TGG CAG CAG CAC TAC CTG CAC Asn Asn Phe His Asn Leu Leu Arg Phe Trp Gln Gln His Tyr Leu His 240 245 250 255	766
AAG GAC AAG GAC AGC ACC TGC CTA GAG AAC AGC TCC TGC ATC AGC TTC Lys Asp Lys Asp Ser Thr Cys Leu Glu Asn Ser Ser Cys Ile Ser Phe 260 265 270	814
TCA TAC TGG AAG GAG ACA GTG TCC ATC CTG TTG AAC CCG GAC CGG CAG Ser Tyr Trp Lys Glu Thr Val Ser Ile Leu Leu Asn Pro Asp Arg Gln 275 280 285	862
TCA CCC TCT GCT CTC GTT AGC TAC ATT GAG GAG CCC TAC ATG GAC ATA Ser Pro Ser Ala Leu Val Ser Tyr Ile Glu Glu Pro Tyr Met Asp Ile 290 295 300	910
GAC AGG GAC TTC ACT GAG GAG TGACCTTGGG CCAGGCCTCG GGAGGCTGCT Asp Arg Asp Phe Thr Glu Glu 305 310	961
GGGCCAGTG? GGGTGAGCGT GGGTACGATG CCACACGCCC TGCCCTGTTC CCGTTCCTCC	1021
CTGCTGCTCT CTGCCTGCCC CAGGTCTTTG GGTACAGGCT TGGTGGGAGG GAAGTCCTAG	1081
AAGCCCTTGG TCCCCCTGGG TCTGAGGGCC CTAGGTCATG GAGAGCCTCA GTCCCCATAA	1141
TGAGGACAGG GTACCATGCC CACCTTTCCT TCAGAACCCT GGGGCCCAGG GCCACCCAGA	1201
GGTAAGAGGA CATTTAGCAT TAGCTCTGTG TGAGCTCCTG CCGGTTTCTT GGCTGTCACT	1261
CAGTCCCAGA GTGGGGAGGA AGATATGGGT GACCCCCACC CCCCATCTGT GAGCCAAGCC	1321
TCCCTTGTC CTGGCCTTTG GACCCAGGCA AAGGCTTCTG AGCCCTGGGC AGGGGTGGTG	1381
GGTACCAGAG AATGCTGCCT TCCCCAAGC CTGCCCTCT GCCTCATTTT CCTGTAGCTC	1441
CTCTGGTTCT GTTTGCTCAT TGGCCGCTGT GTTCATCCAA GGGGGTTCTC CCAGAAGTGA	1501
GGGGCCTTTC CCTCCATCCC TTGGGGCAGG GGGCAGCTGT GCCTGCCCTG CCTCTGCCTG	1561

AGGCAGCCGC TCCTGCCTGA GCCTGGACAT GGGGCCCTTC CTTGTGTTGC CAATTTATTA 1621
 ACAGCAAATA AACCAATTAA ATGGAGACTA TTAAATAACT TTATTTTAAA AATGAAAAAA 1681
 AAAAAAAAAA AAA 1694

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 310 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Leu Lys Ala Asn Ile Pro Glu Val Glu Ala Val Leu Asn Thr Asp
 1 5 10 15
 Arg Ser Leu Val Cys Asp Gly Lys Arg Gly Leu Leu Thr Arg Leu Leu
 20 25 30
 Gln Val Met Lys Lys Glu Pro Ala Glu Ser Ser Phe Arg Phe Trp Gln
 35 40 45
 Ala Arg Ala Val Glu Ser Phe Leu Arg Gly Thr Thr Ser Tyr Ala Asp
 50 55 60
 Gln Met Phe Leu Leu Lys Arg Gly Leu Leu Glu His Ile Leu Tyr Cys
 65 70 75 80
 Ile Val Asp Ser Glu Cys Lys Ser Arg Asp Val Leu Gln Ser Tyr Phe
 85 90 95
 Asp Leu Leu Gly Glu Leu Met Lys Phe Asn Val Asp Ala Phe Lys Arg
 100 105 110
 Phe Asn Lys Tyr Ile Asn Thr Asp Ala Lys Phe Gln Val Phe Leu Lys
 115 120 125
 Gln Ile Asn Ser Ser Leu Val Asp Ser Asn Met Leu Val Arg Cys Val
 130 135 140
 Thr Leu Ser Leu Asp Arg Phe Glu Asn Gln Val Asp Met Lys Val Ala
 145 150 155 160
 Glu Val Leu Ser Glu Cys Arg Leu Leu Ala Tyr Ile Ser Gln Val Pro
 165 170 175
 Thr Gln Met Ser Phe Leu Phe Arg Leu Ile Asn Ile Ile His Val Gln
 180 185 190
 Thr Leu Thr Gln Glu Asn Val Ser Cys Leu Asn Thr Ser Leu Val Ile
 195 200 205
 Leu Met Leu Ala Arg Arg Lys Glu Arg Leu Pro Leu Tyr Leu Arg Leu
 210 215 220
 Leu Gln Arg Met Glu His Ser Lys Lys Tyr Pro Gly Phe Leu Leu Asn
 225 230 235 240
 Asn Phe His Asn Leu Leu Arg Phe Trp Gln Gln His Tyr Leu His Lys
 245 250 255
 Asp Lys Asp Ser Thr Cys Leu Glu Asn Ser Ser Cys Ile Ser Phe Ser

260 265 270
 Tyr Trp Lys Glu Thr Val Ser Ile Leu Leu Asn Pro Asp Arg Gln Ser
 275 280 285
 Pro Ser Ala Leu Val Ser Tyr Ile Glu Glu Pro Tyr Met Asp Ile Asp
 290 295 300
 Arg Asp Phe Thr Glu Glu
 305 310

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2735 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 2..1822

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

G GAG ATC AGT CGG AAG GTG TAC AAG GGA ATG TTA GAC CTC CTC AAG 46
 Glu Ile Ser Arg Lys Val Tyr Lys Gly Met Leu Asp Leu Leu Lys
 1 5 10 15
 TGT ACA GTC CTC AGC TTG GAG CAG TCC TAT GCC CAC GCG GGT CTG GGT 94
 Cys Thr Val Leu Ser Leu Glu Gln Ser Tyr Ala His Ala Gly Leu Gly
 20 25 30
 GGC ATG GCC AGC ATC TTT GGG CTT TTG GAG ATT GCC CAG ACC CAC TAC 142
 Gly Met Ala Ser Ile Phe Gly Leu Leu Glu Ile Ala Gln Thr His Tyr
 35 40 45
 TAT AGT AAA GAA CCA GAC AAG CGG AAG AGA AGT CCA ACA GAA AGT GTA 190
 Tyr Ser Lys Glu Pro Asp Lys Arg Lys Arg Ser Pro Thr Glu Ser Val
 50 55 60
 AAT ACC CCA GTT GGC AAG GAT CCT GGC CTA GCT GGG CGG GGG GAC CCA 238
 Asn Thr Pro Val Gly Lys Asp Pro Gly Leu Ala Gly Arg Gly Asp Pro
 65 70 75
 AAG GCT ATG GCA CAA CTG AGA GTT CCA CAA CTG GGA CCT CGG GCA CCA 286
 Lys Ala Met Ala Gln Leu Arg Val Pro Gln Leu Gly Pro Arg Ala Pro
 80 85 90 95
 AGT GCC ACA GGA AAG GGT CCT AAG GAA CTG GAC ACC AGA AGT TTA AAG 334
 Ser Ala Thr Gly Lys Gly Pro Lys Glu Leu Asp Thr Arg Ser Leu Lys
 100 105 110
 GAA GAA AAT TTT ATA GCA TCT ATT GGG CCT GAA GTA ATC AAA CCT GTC 382
 Glu Glu Asn Phe Ile Ala Ser Ile Gly Pro Glu Val Ile Lys Pro Val
 115 120 125
 TTT GAC CTT GGT GAG ACA GAG GAG AAA AAG TCC CAG ATC AGC GCA GAC 430
 Phe Asp Leu Gly Glu Thr Glu Glu Lys Lys Ser Gln Ile Ser Ala Asp
 130 135 140

AGT GGT GTG AGC CTG ACG TCT AGT TCC CAG AGG ACT GAT CAA GAC TCT Ser Gly Val Ser Leu Thr Ser Ser Ser Gln Arg Thr Asp Gln Asp Ser 145 150 155	478
GTC ATC GGC GTG AGT CCA GCT GTT ATG ATC CGC AGC TCA AGT CAG GAT Val Ile Gly Val Ser Pro Ala Val Met Ile Arg Ser Ser Ser Gln Asp 160 165 170 175	526
TCT GAA GTT AGC ACC GTG GTG AGT AAT AGC TCT GGA GAG ACC CTT GGA Ser Glu Val Ser Thr Val Val Ser Asn Ser Ser Gly Glu Thr Leu Gly 180 185 190	574
GCT GAC AGT GAC TTG AGC AGC AAT GCA GGT GAT GGA CCA GGT GGC GAG Ala Asp Ser Asp Leu Ser Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu 195 200 205	622
GGC AGT GTT CAC CTG GCA AGC TCT CGG GGC ACT TTG TCT GAT AGT GAA Gly Ser Val His Leu Ala Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu 210 215 220	670
ATT GAG ACC AAC TCT GCC ACA AGC ACC ATC TTT GGT AAA GCC CAC AGC Ile Glu Thr Asn Ser Ala Thr Ser Thr Ile Phe Gly Lys Ala His Ser 225 230 235	718
TTG AAG CCA AGC ATA AAG GAG AAG CTG GCA GGC AGC CCC ATT CGT ACT Leu Lys Pro Ser Ile Lys Glu Lys Leu Ala Gly Ser Pro Ile Arg Thr 240 245 250 255	766
TCT GAA GAT GTG AGC CAG CGA GTC TAT CTC TAT GAG GGA CTC CTA GGC Ser Glu Asp Val Ser Gln Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly 260 265 270	814
AAA GAG CGT TCT ACT TTA TGG GAC CAA ATG CAA TTC TGG GAA GAT GCC Lys Glu Arg Ser Thr Leu Trp Asp Gln Met Gln Phe Trp Glu Asp Ala 275 280 285	862
TTC TTA GAT GCT GTG ATG TTG GAG AGA GAA GGG ATG GGT ATG GAC CAG Phe Leu Asp Ala Val Met Leu Glu Arg Glu Gly Met Gly Met Asp Gln 290 295 300	910
GGT CCC CAG GAA ATG ATC GAC AGG TAC CTG TCC CTT GGA GAA CAT GAC Gly Pro Gln Glu Met Ile Asp Arg Tyr Leu Ser Leu Gly Glu His Asp 305 310 315	958
CGG AAG CGC CTG GAA GAT GAT GAA GAT CGC TTG CTG GCC ACA CTT CTG Arg Lys Arg Leu Glu Asp Asp Glu Asp Arg Leu Leu Ala Thr Leu Leu 320 325 330 335	1006
CAC AAC CTC ATC TCC TAC ATG CTG CTG ATG AAG GTA AAT AAG AAT GAC His Asn Leu Ile Ser Tyr Met Leu Leu Met Lys Val Asn Lys Asn Asp 340 345 350	1054
ATC CGC AAG AAG GTG AGG CGC CTA ATG GGA AAG TCG CAC ATT GGG CTT Ile Arg Lys Lys Val Arg Arg Leu Met Gly Lys Ser His Ile Gly Leu 355 360 365	1102
GTG TAC AGC CAG CAA ATC AAT GAG GTG CTT GAT CAG CTG GCG AAC CTG Val Tyr Ser Gln Gln Ile Asn Glu Val Leu Asp Gln Ala Asn Leu 370 375 380	1150
AAT GGA CGC GAT CTC TCT ATC TGG TCC AGT GGC AGC CGG CAC ATG AAG Asn Gly Arg Asp Leu Ser Ile Trp Ser Ser Gly Ser Arg His Met Lys 385 390 395	1198
AAG CAG ACA TTT GTG GTA CAT GCA GGG ACA GAT ACA AAC GGA GAT ATC Lys Gln Thr Phe Val Val His Ala Gly Thr Asp Thr Asn Gly Asp Ile 400 405 410 415	1246

TTT TTC ATG GAG GTG TGC GAT GAC TGT GTG GTG TTG CGT AGT AAC ATC Phe Phe Met Glu Val Cys Asp Asp Cys Val Val Leu Arg Ser Asn Ile 420 425 430	1294
GGA ACA GTG TAT GAG CGC TGG TGG TAC GAG AAG CTC ATC AAC ATG ACC Gly Thr Val Tyr Glu Arg Trp Trp Tyr Glu Lys Leu Ile Asn Met Thr 435 440 445	1342
TAC TGT CCC AAG ACG AAG GTG TTG TGC TTG TGG CGT AGA AAT GGC TCT Tyr Cys Pro Lys Thr Lys Val Leu Cys Leu Trp Arg Arg Asn Gly Ser 450 455 460	1390
GAG ACC CAG CTC AAC AAG TTC TAT ACT AAA AAG TGT CGG GAG CTG TAC Glu Thr Gln Leu Asn Lys Phe Tyr Thr Lys Lys Cys Arg Glu Leu Tyr 465 470 475	1438
TAC TGT GTG AAG GAC AGC ATG GAG CGC GCT GCC GCC CGA CAG CAA AGC Tyr Cys Val Lys Asp Ser Met Glu Arg Ala Ala Ala Arg Gln Gln Ser 480 485 490 495	1486
ATC AAA CCC GGA CCT GAA TTG GGT GGC GAG TTC CCG GTG CAG GAC CTG Ile Lys Pro Gly Pro Glu Leu Gly Gly Glu Phe Pro Val Gln Asp Leu 500 505 510	1534
AAG ACT GGT GAG GGT GGC CTG CTG CAG GTG ACC CTG GAA GGG ATC AAC Lys Thr Gly Glu Gly Gly Leu Leu Gln Val Thr Leu Glu Gly Ile Asn 515 520 525	1582
CTC AAA TTC ATG CAC AAT CAG GTT TTC ATA GAG CTG AAT CAC ATT AAA Leu Lys Phe Met His Asn Gln Val Phe Ile Glu Leu Asn His Ile Lys 530 535 540	1630
AAG TGC AAT ACA GTT CGA GGC GTC TTT GTC CTG GAG GAA TTT GTT CCT Lys Cys Asn Thr Val Arg Gly Val Phe Val Leu Glu Glu Phe Val Pro 545 550 555	1678
GAA ATT AAA GAA GTG GTG AGC CAC AAG TAC AAG ACA CCA ATG GCC CAC Glu Ile Lys Glu Val Val Ser His Lys Tyr Lys Thr Pro Met Ala His 560 565 570 575	1726
GAA ATC TGC TAC TCC GTA TTA TGT CTC TTC TCG TAC GTG GCT GCA GTT Glu Ile Cys Tyr Ser Val Leu Cys Leu Phe Ser Tyr Val Ala Ala Val 580 585 590	1774
CAT AGC AGT GAG GAA GAT CTC AGA ACC CCG CCC CGG CCT GTC TCT AGC His Ser Ser Glu Glu Asp Leu Arg Thr Pro Pro Arg Pro Val Ser Ser 595 600 605	1822
TGATGGAGAG GGGCTACGCA GCTGCCCCAG CCCAGGGCAC GCCCCTGGCC CCTTGCTGTT	1882
CCCAAGTGCA CGATGCTGCT GTGACTGAGG AGTGGATGAT GCTCGTGTGT CCTCTGCAAG	1942
CCCCCTGCTG TGGCTTGGTT GGTTACCGGT TATGTGTCCC TCTGAGTGTG TCTTGAGCGT	2002
GTCCACCTTC TCCCTCTCCA CTCCCAGAAG ACCAACTGC CTTCCCCTCA GGGCTCAAGA	2062
ATGTGTACAG TCTGTGGGGC CGGTGTGAAC CCACTATTTT GTGTCCTTGA GACATTTGTG	2122
TTGTGGTTCC TTGTCCTTGT CCCTGGCGTT ATAAGTGTCC ACTGCAAGAG TCTGGCTCTC	2182
CCTTCTCTGT GACCCGGCAT GACTGGGCGC CTGGAGCAGT TTCACTCTGT GAGGAGTGAG	2242
GGAACCTGG GGCTCACCT CTCAGAGGAA GGGCACAGAG AGGAAGGGAA GAATTGGGGG	2302
GCAGCCGGAG TGAGTGGCAG CCTCCCTGCT TCCTTCTGCA TTCCAAGCC GGCAGCTACT	2362
CCCCAGGGCC CGCASTGTTG GCTGCTGCCT GCCACAGCCT CTGTGACTGC AGTGGAGCGG	2422

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CGAATTCCTT GTGGCCTGCC ACGCCTTCGG CATCAGAGGA TGGAGTGGTC GAGGCTAGTG      2482
GAGTCCCAGG GACCGCTGGC TGCTCTGCCT GAGCATCAGG GAGGGGGCAG GAAAGACCAA      2542
GCTGGGTTTG CACATCTGTC TGCAGGCTGT CTCTCCAGGC ACGGGGTGTC AGGAGGGAGA      2602
GACAGCCTGG GTATGGGCAA GAAATGACTG TAAATATTTC AGCCCCACAT TATTTATAGA      2662
AAATGTACAG TTGTGTGAAT GTGAAATAAA TGTCCTCAAC TCCCAAAAAA AAAAAAAAAA      2722
AAAAAAAAAA AAA                                                              2735

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(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 607 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Glu Ile Ser Arg Lys Val Tyr Lys Gly Met Leu Asp Leu Leu Lys Cys
 1             5             10
Thr Val Leu Ser Leu Glu Gln Ser Tyr Ala His Ala Gly Leu Gly Gly
 20            25            30
Met Ala Ser Ile Phe Gly Leu Leu Glu Ile Ala Gln Thr His Tyr Tyr
 35            40            45
Ser Lys Glu Pro Asp Lys Arg Lys Arg Ser Pro Thr Glu Ser Val Asn
 50            55            60
Thr Pro Val Gly Lys Asp Pro Gly Leu Ala Gly Arg Gly Asp Pro Lys
 65            70            75
Ala Met Ala Gln Leu Arg Val Pro Gln Leu Gly Pro Arg Ala Pro Ser
 85            90            95
Ala Thr Gly Lys Gly Pro Lys Glu Leu Asp Thr Arg Ser Leu Lys Glu
100           105           110
Glu Asn Phe Ile Ala Ser Ile Gly Pro Glu Val Ile Lys Pro Val Phe
115           120           125
Asp Leu Gly Glu Thr Glu Glu Lys Lys Ser Gln Ile Ser Ala Asp Ser
130           135           140
Gly Val Ser Leu Thr Ser Ser Ser Gln Arg Thr Asp Gln Asp Ser Val
145           150           155           160
Ile Gly Val Ser Pro Ala Val Met Ile Arg Ser Ser Ser Gln Asp Ser
165           170           175
Glu Val Ser Thr Val Val Ser Asn Ser Ser Gly Glu Thr Leu Gly Ala
180           185           190
Asp Ser Asp Leu Ser Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu Gly
195           200           205
Ser Val His Leu Ala Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu Ile
210           215           220
Glu Thr Asn Ser Ala Thr Ser Thr Ile Phe Gly Lys Ala His Ser Leu

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15

Ser Ser Glu Glu Asp Leu Arg Thr Pro Pro Arg Pro Val Ser Ser
 595 600 605

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3225 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 3..2846

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CC CAG ACT CGC CCC GCC CCA GAG ACT GCG CCT GCG CGG GCA CGA GAC	47
Gln Thr Arg Pro Ala Pro Glu Thr Ala Pro Ala Arg Ala Arg Asp	
1 5 10 15	
ACC CTC TCC GCG ATG ACT GCC AGC TCA GTG GAG CAG CTG CGG AAG GAG	95
Thr Leu Ser Ala Met Thr Ala Ser Ser Val Glu Gln Leu Arg Lys Glu	
20 25 30	
GGC AAT GAG CTG TTC AAA TGT GGA GAC TAC GGG GGC GCC CTG GCG GCC	143
Gly Asn Glu Leu Phe Lys Cys Gly Asp Tyr Gly Gly Ala Leu Ala Ala	
35 40 45	
TAC ACT CAG GCC CTG GGT CTG GAC GCG ACG CCC CAG GAC CAG GCC GTT	191
Tyr Thr Gln Ala Leu Gly Leu Asp Ala Thr Pro Gln Asp Gln Ala Val	
50 55 60	
CTG CAC CGG AAC CGG GCC GCC TGC CAC CTC AAG CTG GAA GAT TAC GAC	239
Leu His Arg Asn Arg Ala Ala Cys His Leu Lys Leu Glu Asp Tyr Asp	
65 70 75	
AAA GCA GAA ACA GAG GCA TCC AAA GCC ATT GAA AAG GAT GGT GGG GAT	287
Lys Ala Glu Thr Glu Ala Ser Lys Ala Ile Glu Lys Asp Gly Gly Asp	
80 85 90 95	
GTC AAA GCA CTC TAC CGG CGG AGC CAA GCC CTA GAG AAG CTG GGC CGC	335
Val Lys Ala Leu Tyr Arg Arg Ser Gln Ala Leu Glu Lys Leu Gly Arg	
100 105 110	
CTG GAC CAG GCT GTC CTT GAC CTG CAG AGA TGT GTG AGC TTG GAG CCC	383
Leu Asp Gln Ala Val Leu Asp Leu Gln Arg Cys Val Ser Leu Glu Pro	
115 120 125	
AAG AAC AAA GTT TTC CAG GAG GCC TTG CGG AAC ATC GGG GGC CAG ATT	431
Lys Asn Lys Val Phe Gln Glu Ala Leu Arg Asn Ile Gly Gly Gln Ile	
130 135 140	
CAG GAG AAG GTG CGA TAC ATG TCC TCG ACG GAT GCC AAA GTG GAA CAG	479
Gln Glu Lys Val Arg Tyr Met Ser Ser Thr Asp Ala Lys Val Glu Gln	
145 150 155	

ATG TTT CAG ATA CTG TTG GAC CCA GAA GAG AAG GGC ACT GAG AAA AAG Met Phe Gln Ile Leu Leu Asp Pro Glu Glu Lys Gly Thr Glu Lys Lys 160 165 170 175	527
CAA AAG GCT TCT CAG AAC CTG GTG GTG CTG GCC AGG GAG GAT GCT GGA Gln Lys Ala Ser Gln Asn Leu Val Val Leu Ala Arg Glu Asp Ala Gly 180 185 190	575
GCG GAG AAG ATC TTC CGG AGT AAT GGG GTT CAG CTC TTG CAA CGT TTA Ala Glu Lys Ile Phe Arg Ser Asn Gly Val Gln Leu Leu Gln Arg Leu 195 200 205	623
CTG GAC ATG GGA GAG ACT GAC CTC ATG CTG GCG GCT CTG CGT ACG CTG Leu Asp Met Gly Glu Thr Asp Leu Met Leu Ala Ala Leu Arg Thr Leu 210 215 220	671
GTT GGC ATT TGC TCT GAG CAT CAG TCA CGG ACA GTG GCA ACC CTG AGC Val Gly Ile Cys Ser Glu His Gln Ser Arg Thr Val Ala Thr Leu Ser 225 230 235	719
ATA CTG GGA ACT CGG CGA GTA GTC TCC ATC CTG GGC GTG GAA ACC CAG Ile Leu Gly Thr Arg Arg Val Val Ser Ile Leu Gly Val Glu Ser Gln 240 245 250 255	767
GCT GTG TCC CTG GCT GCC TGC CAC CTG CTG CAG GTT ATG TTT GAT GCC Ala Val Ser Leu Ala Ala Cys His Leu Leu Gln Val Met Phe Asp Ala 260 265 270	815
CTC AAG GAA GGT GTC AAA AAA GGC TTC CGA GGC AAA GAA GGT GCC ATC Leu Lys Glu Gly Val Lys Lys Gly Phe Arg Gly Lys Glu Gly Ala Ile 275 280 285	863
ATT GTG GAT CCT GCC CGG GAG CTG AAG GTC CTC ATC AGT AAC CTC TTA Ile Val Asp Pro Ala Arg Glu Leu Lys Val Leu Ile Ser Asn Leu Leu 290 295 300	911
GAT CTG CTG ACA GAG GTG GGG GTC TCT GGC CAA GGC CGA GAC AAT GCC Asp Leu Leu Thr Glu Val Gly Val Ser Gly Gln Gly Arg Asp Asn Ala 305 310 315	959
CTG ACC CTC CTG ATT AAA GCG GTG CCC CGG AAG TCT CTC AAG GAC CCC Leu Thr Leu Leu Ile Lys Ala Val Pro Arg Lys Ser Leu Lys Asp Pro 320 325 330 335	1007
AAC AAC AGC CTC ACC CTC TGG GTC ATC GAC CAA GGT CTG AAA AAG ATT Asn Asn Ser Leu Thr Leu Trp Val Ile Asp Gln Gly Leu Lys Lys Ile 340 345 350	1055
TTG GAA GTG GGG GGC TCT CTA CAG GAC CCT CCT GGG GAG CTC GCA GTG Leu Glu Val Gly Gly Ser Leu Gln Asp Pro Pro Gly Glu Leu Ala Val 355 360 365	1103
ACC GCA AAC AGC CGC ATG AGC GCC TCT ATT CTC CTC AGC AAG CTC TTT Thr Ala Asn Ser Arg Met Ser Ala Ser Ile Leu Leu Ser Lys Leu Phe 370 375 380	1151
GAT GAC CTC AAG TGT GAT GCG GAG AGG GAG AAT TTC CAC AGA CTT TGT Asp Asp Leu Lys Cys Asp Ala Glu Arg Glu Asn Phe His Arg Leu Cys 385 390 395	1199
GAA AAC TAC ATC AAG AGC TGG TTT GAG GGC CAA GGG CTG GCC GGG AAG Glu Asn Tyr Ile Lys Ser Trp Phe Glu Gly Gln Gly Leu Ala Gly Lys 400 405 410 415	1247
CTA CGG GCC ATC CAG ACG GTG TCC TGC CTC CTG CAG GGC CCA TGT GAC Leu Arg Ala Ile Gln Thr Val Ser Cys Leu Leu Gln Gly Pro Cys Asp 420 425 430	1295

GCT	GGC	AAC	CGG	GCC	TTG	GAG	CTG	AGC	GGT	GTC	ATG	GAG	AGT	GTG	ATT	1343
Ala	Gly	Asn	Arg	Ala	Leu	Glu	Leu	Ser	Gly	Val	Met	Glu	Ser	Val	Ile	
			435					440					445			
GCT	CTG	TGT	GCC	TCT	GAG	CAG	GAG	GAG	GAG	CAG	CTG	GTG	GCC	GTG	GAG	1391
Ala	Leu	Cys	Ala	Ser	Glu	Gln	Glu	Glu	Glu	Gln	Leu	Val	Ala	Val	Glu	
		450					455					460				
GCT	CTG	ATC	CAT	GCA	GCC	GGC	AAG	GCT	AAG	CGG	GCC	TCA	TTC	ATC	ACT	1439
Ala	Leu	Ile	His	Ala	Ala	Gly	Lys	Ala	Lys	Arg	Ala	Ser	Phe	Ile	Thr	
	465					470					475					
GCC	AAT	GGT	GTC	TCG	CTG	CTG	AAG	GAC	CTA	TAT	AAG	TGC	AGC	GAG	AAG	1487
Ala	Asn	Gly	Val	Ser	Leu	Leu	Lys	Asp	Leu	Tyr	Lys	Cys	Ser	Glu	Lys	
480					485					490					495	
GAC	AGC	ATC	CGC	ATC	CGG	GCG	CTA	GTG	GGA	CTC	TGT	AAG	CTC	GGT	TCG	1535
Asp	Ser	Ile	Arg	Ile	Arg	Ala	Leu	Val	Gly	Leu	Cys	Lys	Leu	Gly	Ser	
				500					505					510		
GCT	GGA	GGG	ACT	GAC	TTC	AGC	ATG	AAG	CAG	TTT	GCT	GAA	GGC	TCC	ACT	1583
Ala	Gly	Gly	Thr	Asp	Phe	Ser	Met	Lys	Gln	Phe	Ala	Glu	Gly	Ser	Thr	
			515					520					525			
CTC	AAA	CTG	GCT	AAG	CAG	TGT	CGA	AAG	TGG	CTG	TGC	AAT	GAC	CAG	ATC	1631
Leu	Lys	Leu	Ala	Lys	Gln	Cys	Arg	Lys	Trp	Leu	Cys	Asn	Asp	Gln	Ile	
		530					535					540				
GAC	GCA	GGC	ACT	CGG	CGC	TGG	GCA	GTG	GAG	GGC	CTG	GCT	TAC	CTG	ACC	1679
Asp	Ala	Gly	Thr	Arg	Arg	Trp	Ala	Val	Glu	Gly	Leu	Ala	Tyr	Leu	Thr	
		545				550					555					
TTT	GAT	GCC	GAC	GTG	AAG	GAA	GAG	TTT	GTG	GAG	GAT	GCG	GCT	GCT	CTG	1727
Phe	Asp	Ala	Asp	Val	Lys	Glu	Glu	Phe	Val	Glu	Asp	Ala	Ala	Ala	Leu	
560					565					570					575	
AAA	GCT	CTG	TTC	CAG	CTC	AGC	AGG	TTG	GAG	GAG	AGG	TCA	GTG	CTC	TTT	1775
Lys	Ala	Leu	Phe	Gln	Leu	Ser	Arg	Leu	Glu	Glu	Arg	Ser	Val	Leu	Phe	
				580					585					590		
GCG	GTG	GCC	TCA	GCG	CTG	GTG	AAC	TGC	ACC	AAC	AGC	TAT	GAC	TAC	GAG	1823
Ala	Val	Ala	Ser	Ala	Leu	Val	Asn	Cys	Thr	Asn	Ser	Tyr	Asp	Tyr	Glu	
			595					600					605			
GAG	CCC	GAC	CCC	AAG	ATG	GTG	GAG	CTG	GCC	AAG	TAT	GCC	AAG	CAG	CAT	1871
Glu	Pro	Asp	Pro	Lys	Met	Val	Glu	Leu	Ala	Lys	Tyr	Ala	Lys	Gln	His	
		610					615					620				
GTG	CCC	GAG	CAG	CAC	CCC	AAG	GAC	AAG	CCA	AGC	TTC	GTG	CGG	GCT	CGG	1919
Val	Pro	Glu	Gln	His	Pro	Lys	Asp	Lys	Pro	Ser	Phe	Val	Arg	Ala	Arg	
		625				630					635					
GTG	AAG	AAG	CTG	CTG	GCA	GCG	GGT	GTG	GTG	TCG	GCC	ATG	GTG	TGC	ATG	1967
Val	Lys	Lys	Leu	Leu	Ala	Ala	Gly	Val	Val	Ser	Ala	Met	Val	Cys	Met	
640					645					650					655	
GTG	AAG	ACG	GAG	AGC	CCT	GTG	CTG	ACC	AGT	TCC	TGC	AGA	GAG	CTG	CTC	2015
Val	Lys	Thr	Glu	Ser	Pro	Val	Leu	Thr	Ser	Ser	Cys	Arg	Glu	Leu	Leu	
				660					665					670		
TCC	AGG	GTC	TTC	TTG	GCT	TTA	GTG	GAA	GAG	GTA	GAG	GAC	CGA	GGC	ACT	2063
Ser	Arg	Val	Phe	Leu	Ala	Leu	Val	Glu	Glu	Val	Glu	Asp	Arg	Gly	Thr	
			675					680					685			
GTG	GTT	GCC	CAG	GGA	GGC	GGC	AGG	GCG	CTG	ATC	CCG	CTG	GCC	CTG	GAA	2111
Val	Val	Ala	Gln	Gly	Gly	Gly	Arg	Ala	Leu	Ile	Pro	Leu	Ala	Leu	Glu	
		690					695					700				

GGC ACG GAC GTG GGG CAG ACA AAG GCA GCC CAG GCC CTT GCC AAG CTC Gly Thr Asp Val Gly Gln Thr Lys Ala Ala Gln Ala Leu Ala Lys Leu 705 710 715	2159
ACC ATC ACC TCC AAC CCG GAG ATG ACC TTC CCT GGC GAG CGG ATC TAT Thr Ile Thr Ser Asn Pro Glu Met Thr Phe Pro Gly Glu Arg Ile Tyr 720 725 730 735	2207
GAG GTG GTC CGG CCC CTC GTC TCC CTG TTG CAC CTC AAC TGC TCA GGC Glu Val Val Arg Pro Leu Val Ser Leu Leu His Leu Asn Cys Ser Gly 740 745 750	2255
CTG CAG AAC TTC GAG GCG CTC ATG GCC CTA ACA AAC CTG GCT GGG ATC Leu Gln Asn Phe Glu Ala Leu Met Ala Leu Thr Asn Leu Ala Gly Ile 755 760 765	2303
AGC GAG AGG CTC CGG CAG AAG ATC CTG AAG GAG AAG GCT GTG CCC ATG Ser Glu Arg Leu Arg Gln Lys Ile Leu Lys Glu Lys Ala Val Pro Met 770 775 780	2351
ATA GAA GGC TAC ATG TTT GAG GAG CAT GAG ATG ATC CGC CGG GCA GCC Ile Glu Gly Tyr Met Phe Glu Glu His Glu Met Ile Arg Arg Ala Ala 785 790 795	2399
ACG GAG TGC ATG TGT AAC TTG GCC ATG AGC AAG GAG GTG CAG GAC CTC Thr Glu Cys Met Cys Asn Leu Ala Met Ser Lys Glu Val Gln Asp Leu 800 805 810 815	2447
TTC GAA GCC CAG GGC AAT GAC CGA CTG AAG CTG CTG GTG CTG TAC AGT Phe Glu Ala Gln Gly Asn Asp Arg Leu Lys Leu Leu Val Leu Tyr Ser 820 825 830	2495
GGA GAG GAT GAT GAG CTG CTA CAG CGG GCA GCT GCC GGG GGC TTG GCC Gly Glu Asp Asp Glu Leu Leu Gln Arg Ala Ala Ala Gly Gly Leu Ala 835 840 845	2543
ATG CTT ACC TCC ATG CGG CCC ACG CTC TGC AGC CGC ATT CCC CAA GTG Met Leu Thr Ser Met Arg Pro Thr Leu Cys Ser Arg Ile Pro Gln Val 850 855 860	2591
ACC ACA CAC TGG CTG GAG ATC CTG CAG GCC CTG CTT CTG AGC TCC AAC Thr Thr His Trp Leu Glu Ile Leu Gln Ala Leu Leu Leu Ser Ser Asn 865 870 875	2639
CAG GAG CTG CAG CAC CGG GGT GCT GTG GTG GTG CTG AAC ATG GTG GAG Gln Glu Leu Gln His Arg Gly Ala Val Val Val Leu Asn Met Val Glu 880 885 890 895	2687
GCC TCG AGG GAG ATT GCC AGC ACC CTG ATG GAG AGT GAG ATG ATG GAG Ala Ser Arg Glu Ile Ala Ser Thr Leu Met Glu Ser Glu Met Met Glu 900 905 910	2735
ATC TTG TCA GTG CTA GCT AAG GGT GAC CAC AGC CCT GTC ACA AGG GCT Ile Leu Ser Val Leu Ala Lys Gly Asp His Ser Pro Val Thr Arg Ala 915 920 925	2783
GCT GCA GCC TGC CTG GAC AAA GCA GTG GAA TAT GGG CTT ATC CAA CCC Ala Ala Ala Cys Leu Asp Lys Ala Val Glu Tyr Gly Leu Ile Gln Pro 930 935 940	2831
AAC CAA GAT GGA GAG TGAGGGGGTT GTCCCTGGGC CCAAGGCTCA TGCACACGCT Asn Gln Asp Gly Glu 945	2886
ACCTATTGTG GCACGGAGAG TAAGGACGGA AGCAGCTTTG GCTGGTGGTG GCTGGCATTC	2946
CCAATACTCT TGCCCATCCT CGCTTGCTGC CCTAGGATGT CCTCTGTTCT GAGTCAGTCC	3006

CCACGTTTCAG TCACACAGCC CTGCTTGGCC AGCACTGCCT GCAGCCTCAC TCAGAGGGGG 3066
 CCTTTTTCTG TACTACTGTA GTCAGCTGGG AATGGGGAAG GTGCATCCCA ACACAGCCTG 3126
 TGGATCCTGG GGCATTTGGA AGGGCGCACA CATCAGCAGC CTCACCAGCT GTGAGCCTGC 3186
 TATCAGGCCT GCCCCTCCAA TAAAAGTGTG TAGAACTCC 3225

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 948 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Thr Arg Pro Ala Pro Glu Thr Ala Pro Ala Arg Ala Arg Asp Thr
 1 5 10 15
 Leu Ser Ala Met Thr Ala Ser Ser Val Glu Gln Leu Arg Lys Glu Gly
 20 25 30
 Asn Glu Leu Phe Lys Cys Gly Asp Tyr Gly Gly Ala Leu Ala Ala Tyr
 35 40 45
 Thr Gln Ala Leu Gly Leu Asp Ala Thr Pro Gln Asp Gln Ala Val Leu
 50 55 60
 His Arg Asn Arg Ala Ala Cys His Leu Lys Leu Glu Asp Tyr Asp Lys
 65 70 75 80
 Ala Glu Thr Glu Ala Ser Lys Ala Ile Glu Lys Asp Gly Gly Asp Val
 85 90 95
 Lys Ala Leu Tyr Arg Arg Ser Gln Ala Leu Glu Lys Leu Gly Arg Leu
 100 105 110
 Asp Gln Ala Val Leu Asp Leu Gln Arg Cys Val Ser Leu Glu Pro Lys
 115 120 125
 Asn Lys Val Phe Gln Glu Ala Leu Arg Asn Ile Gly Gly Gln Ile Gln
 130 135 140
 Glu Lys Val Arg Tyr Met Ser Ser Thr Asp Ala Lys Val Glu Gln Met
 145 150 155 160
 Phe Gln Ile Leu Leu Asp Pro Glu Glu Lys Gly Thr Glu Lys Lys Gln
 165 170 175
 Lys Ala Ser Gln Asn Leu Val Val Leu Ala Arg Glu Asp Ala Gly Ala
 180 185 190
 Glu Lys Ile Phe Arg Ser Asn Gly Val Gln Leu Leu Gln Arg Leu Leu
 195 200 205
 Asp Met Gly Glu Thr Asp Leu Met Leu Ala Ala Leu Arg Thr Leu Val
 210 215 220
 Gly Ile Cys Ser Glu His Gln Ser Arg Thr Val Ala Thr Leu Ser Ile
 225 230 235 240
 Leu Gly Thr Arg Arg Val Val Ser Ile Leu Gly Val Glu Ser Gln Ala
 245 250 255

Val Ser Leu Ala Ala Cys His Leu Leu Gln Val Met Phe Asp Ala Leu
 260 265 270
 Lys Glu Gly Val Lys Lys Gly Phe Arg Gly Lys Glu Gly Ala Ile Ile
 275 280 285
 Val Asp Pro Ala Arg Glu Leu Lys Val Leu Ile Ser Asn Leu Leu Asp
 290 295 300
 Leu Leu Thr Glu Val Gly Val Ser Gly Gln Gly Arg Asp Asn Ala Leu
 305 310 315 320
 Thr Leu Leu Ile Lys Ala Val Pro Arg Lys Ser Leu Lys Asp Pro Asn
 325 330 335
 Asn Ser Leu Thr Leu Trp Val Ile Asp Gln Gly Leu Lys Lys Ile Leu
 340 345 350
 Glu Val Gly Gly Ser Leu Gln Asp Pro Pro Gly Glu Leu Ala Val Thr
 355 360 365
 Ala Asn Ser Arg Met Ser-Ala-Ser Ile Leu Leu Ser Lys Leu Phe Asp
 370 375 380
 Asp Leu Lys Cys Asp Ala Glu Arg Glu Asn Phe His Arg Leu Cys Glu
 385 390 395 400
 Asn Tyr Ile Lys Ser Trp Phe Glu Gly Gln Gly Leu Ala Gly Lys Leu
 405 410 415
 Arg Ala Ile Gln Thr Val Ser Cys Leu Leu Gln Gly Pro Cys Asp Ala
 420 425 430
 Gly Asn Arg Ala Leu Glu Leu Ser Gly Val Met Glu Ser Val Ile Ala
 435 440 445
 Leu Cys Ala Ser Glu Gln Glu Glu Glu Gln Leu Val Ala Val Glu Ala
 450 455 460
 Leu Ile His Ala Ala Gly Lys Ala Lys Arg Ala Ser Phe Ile Thr Ala
 465 470 475 480
 Asn Gly Val Ser Leu Leu Lys Asp Leu Tyr Lys Cys Ser Glu Lys Asp
 485 490 495
 Ser Ile Arg Ile Arg Ala Leu Val Gly Leu Cys Lys Leu Gly Ser Ala
 500 505 510
 Gly Gly Thr Asp Phe Ser Met Lys Gln Phe Ala Glu Gly Ser Thr Leu
 515 520 525
 Lys Leu Ala Lys Gln Cys Arg Lys Trp Leu Cys Asn Asp Gln Ile Asp
 530 535 540
 Ala Gly Thr Arg Arg Trp Ala Val Glu Gly Leu Ala Tyr Leu Thr Phe
 545 550 555 560
 Asp Ala Asp Val Lys Glu Glu Phe Val Glu Asp Ala Ala Ala Leu Lys
 565 570 575
 Ala Leu Phe Gln Leu Ser Arg Leu Glu Glu Arg Ser Val Leu Phe Ala
 580 585 590
 Val Ala Ser Ala Leu Val Asn Cys Thr Asn Ser Tyr Asp Tyr Glu Glu
 595 600 605
 Pro Asp Pro Lys Met Val Glu Leu Ala Lys Tyr Ala Lys Gln His Val

610	615	620
Pro Glu Gln His 625	Pro Lys Asp Lys 630	Pro Ser Phe Val Arg Ala Arg Val 635 640
Lys Lys Leu Leu 645	Ala Ala Gly Val Val 650	Ser Ala Met Val Cys Met Val 655
Lys Thr Glu Ser 660	Pro Val Leu Thr 665	Ser Ser Cys Arg Glu Leu Leu Ser 670
Arg Val Phe Leu 675	Ala Leu Val Glu Glu Val 680	Glu Asp Arg Gly Thr Val 685
Val Ala Gln Gly Gly 690	Gly Arg Ala Leu Ile 695	Pro Leu Ala Leu Glu Gly 700
Thr Asp Val Gly Gln 705	Thr Lys Ala Ala 710	Gln Ala Leu Ala Lys Leu Thr 715 720
Ile Thr Ser Asn 725	Pro Glu Met Thr Phe 730	Pro Gly Glu Arg Ile Tyr Glu 735
Val Val Arg Pro 740	Leu Val Ser Leu Leu 745	His Leu Asn Cys Ser Gly Leu 750
Gln Asn Phe Glu 755	Ala Leu Met Ala Leu Thr 760	Asn Leu Ala Gly Ile Ser 765
Glu Arg Leu Arg 770	Gln Lys Ile Leu Lys 775	Glu Lys Ala Val Pro Met Ile 780
Glu Gly Tyr Met 785	Phe Glu Glu His Glu 790	Met Ile Arg Arg Ala Ala Thr 795 800
Glu Cys Met Cys 805	Asn Leu Ala Met Ser 810	Lys Glu Val Gln Asp Leu Phe 815
Glu Ala Gln Gly 820	Asn Asp Arg Leu Lys 825	Leu Leu Val Leu Tyr Ser Gly 830
Glu Asp Asp Glu 835	Leu Leu Gln Arg Ala 840	Ala Ala Gly Gly Leu Ala Met 845
Leu Thr Ser Met 850	Arg Pro Thr Leu Cys 855	Ser Arg Ile Pro Gln Val Thr 860
Thr His Trp Leu 865	Glu Ile Leu Gln Ala 870	Leu Leu Leu Ser Ser Asn Gln 875 880
Glu Leu Gln His 885	Arg Gly Ala Val Val 890	Val Leu Asn Met Val Glu Ala 895
Ser Arg Glu Ile 900	Ala Ser Thr Leu Met 905	Glu Ser Glu Met Met Glu Ile 910
Leu Ser Val Leu 915	Ala Lys Gly Asp His 920	Ser Pro Val Thr Arg Ala Ala 925
Ala Ala Cys Leu 930	Asp Lys Ala Val Glu 935	Tyr Gly Leu Ile Gln Pro Asn 940
Gln Asp Gly Glu 945		

CLAIMS

What is claimed is:

1. A composition comprising an isolated polynucleotide encoding a protein having TNF-R1-DD ligand protein activity.

5 2. The composition of claim 1 wherein said polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;

10 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2; and

15 (e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

3. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

20 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3;

25 (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4; and

30 (e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

4. A composition of claim 1 wherein said polynucleotide is operably linked to an expression control sequence.

5. A host cell transformed with a composition of claim 4.

5

6. The host cell of claim 5, wherein said cell is a mammalian cell.

7. A process for producing an TNF-R1-DD ligand protein, which comprises:

10 (a) growing a culture of the host cell of claim 5 in a suitable culture medium; and

(b) purifying the TNF-R1-DD ligand protein from the culture.

8. A composition comprising a protein having TNF-R1-DD ligand protein activity.

15

9. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2; and

20 (b) fragments of the amino acid sequence of SEQ ID NO:2; said protein being substantially free from other mammalian proteins.

10. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

25 (a) the amino acid sequence of SEQ ID NO:4; and

(b) fragments of the amino acid sequence of SEQ ID NO:4;

said protein being substantially free from other mammalian proteins.

11. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

30

(a) the amino acid sequence of SEQ ID NO:6; and

(b) fragments of the amino acid sequence of SEQ ID NO:6;

said protein being substantially free from other mammalian proteins.

12. The composition of claim 8, further comprising a pharmaceutically acceptable carrier.

5

13. A composition comprising an antibody which specifically reacts with the TNF-R1-DD ligand protein of claim 8.

10 14. A method of identifying an inhibitor of TNF-R death domain binding which comprises:

(a) combining an TNF-R death domain protein with a composition of claim 8, said combination forming a first binding mixture;

15 (b) measuring the amount of binding between the TNF-R death domain protein and the TNF-R1-DD ligand protein in the first binding mixture;

(c) combining a compound with the TNF-R death domain protein and an TNF-R1-DD ligand protein to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

20 (e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the amount of binding of the second binding mixture occurs.

25 15. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) fragments of the amino acid sequence of SEQ ID NO:2;

(c) the amino acid sequence of SEQ ID NO:4;

- (d) fragments of the amino acid sequence of SEQ ID NO:4;
- (e) the amino acid sequence of SEQ ID NO:6;
- (f) fragments of the amino acid sequence of SEQ ID NO:6;
- (g) the amino acid sequence of SEQ ID NO:8; and
- 5 (h) fragments of the amino acid sequence of SEQ ID NO:8.

16. A method of preventing or ameliorating an inflammatory condition which comprises administering a therapeutically effective amount of a composition of claim 12.

10

17. TNF-R1-DD ligand protein produced according to the method of claim 7.

18. A method of inhibiting TNF-R death domain binding comprising
15 administering a therapeutically effective amount of a composition of claim 12.

19. A method of preventing or ameliorating an inflammatory condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a
20 protein selected from the group consisting of IGFBP-5 and fragments thereof having TNF-R1-DD ligand protein activity.

20. A method of inhibiting TNF-R death domain binding comprising administering to a mammalian subject a therapeutically effective amount of a
25 composition comprising a pharmaceutically acceptable carrier and a protein selected from the group consisting of IGFBP-5 and fragments thereof having TNF-R1-DD ligand protein activity.

21. A composition comprising an inhibitor identified according to the
30 method of claim 14.

22. The composition of claim 21 further comprising a pharmaceutically acceptable carrier.

23. A method of preventing or ameliorating an inflammatory condition comprising administering to a mammalian subject a therapeutically effective amount of the composition of claim 22.

24. A method of inhibiting TNF-R death domain binding comprising administering to a mammalian subject a therapeutically effective amount of the composition of claim 22.

25. A composition comprising a pharmaceutically acceptable carrier and a protein selected from the group consisting of IGFBP-5 and fragments thereof having TNF-R1-DD ligand protein activity.

26. A method of identifying an inhibitor of TNF-R death domain binding which comprises:

(a) transforming a cell with a first polynucleotide encoding an TNF-R death domain protein, a second polynucleotide encoding an TNF-R1-DD ligand protein, and at least one reporter gene, wherein the expression of the reporter gene is regulated by the binding of the TNF-R1-DD ligand protein encoded by the second polynucleotide to the TNF-R death domain protein encoded by the first polynucleotide;

(b) growing the cell in the presence of and in the absence of a compound; and

(c) comparing the degree of expression of the reporter gene in the presence of and in the absence of the compound;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the degree of expression of the reporter gene occurs.

27. The method of claim 26 wherein the second polynucleotide is selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1, which encodes a protein having TNF-R1-DD ligand protein activity;
- (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;
- (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 and having TNF-R1-DD ligand protein activity;
- (e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;
- (f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3, which encodes a protein having TNF-R1-DD ligand protein activity;
- (g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;
- (h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 and having TNF-R1-DD ligand protein activity;
- (i) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 2 to nucleotide 559;
- (j) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5, which encodes a protein having TNF-R1-DD ligand protein activity;
- (k) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:6;
- (l) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 and having TNF-R1-DD ligand protein activity;
- (m) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 57 to nucleotide 875;

(n) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7, which encodes a protein having TNF-R1-DD ligand protein activity;

5 (o) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:8;

(p) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 and having TNF-R1-DD ligand protein activity; and

10 (q) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(p), which encodes a protein having TNF-R1-DD ligand protein activity.

28. The method of claim 26 wherein the cell is a yeast cell.

15 29. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

20 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10; and

25 (e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

30. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

30 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12;

5 (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

10 31. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10; and

(b) fragments of the amino acid sequence of SEQ ID NO:10;
said protein being substantially free from other mammalian proteins.

15

32. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:12; and

(b) fragments of the amino acid sequence of SEQ ID NO:12;
20 said protein being substantially free from other mammalian proteins.

33. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10;

25 (b) fragments of the amino acid sequence of SEQ ID NO:10;

(c) the amino acid sequence of SEQ ID NO:12; and

(d) fragments of the amino acid sequence of SEQ ID NO:12.

34. The method of claim 26 wherein the second polynucleotide is selected
30 from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9, which encodes a protein having TNF-R1-DD ligand protein activity;

5 (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 and having TNF-R1-DD ligand protein activity;

10 (e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;

(f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11, which encodes a protein having TNF-R1-DD ligand protein activity;

15 (g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12; and

(h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 and having TNF-R1-DD ligand protein activity; and

20 (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), which encodes a protein having TNF-R1-DD ligand protein activity.

35. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

25 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 3 to nucleotide 2846;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13;

30 (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14; and

Fig. 1

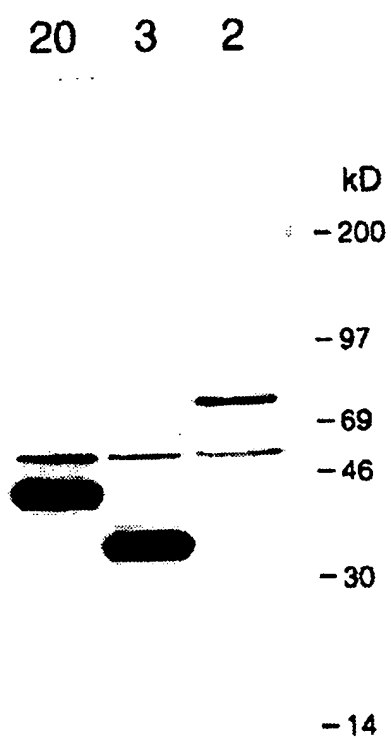


Fig. 2

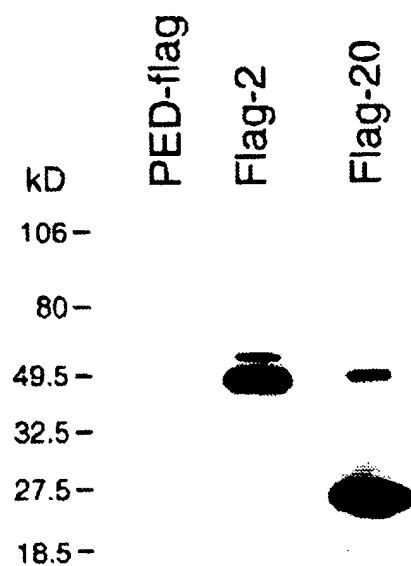


Fig. 3A

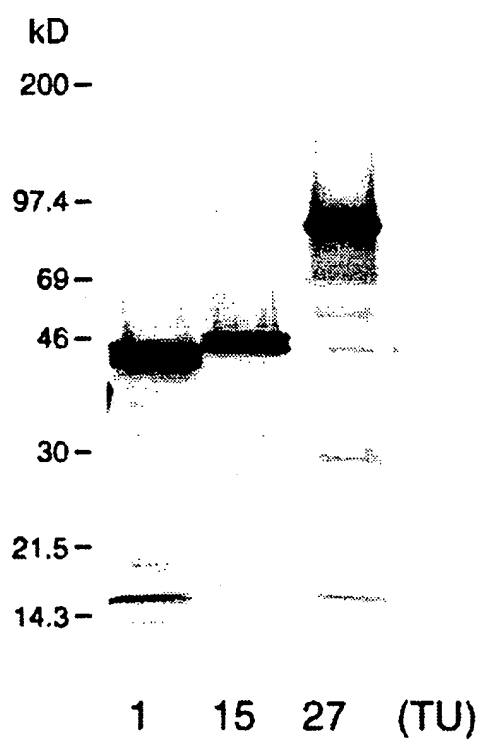


Fig. 3B

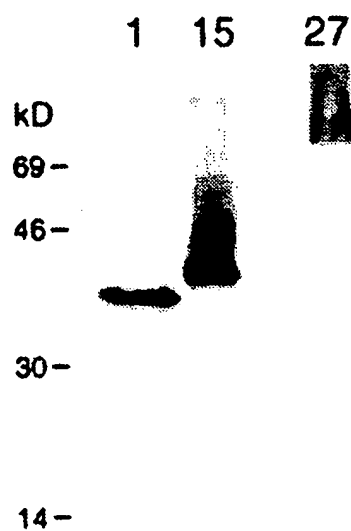


Fig. 4

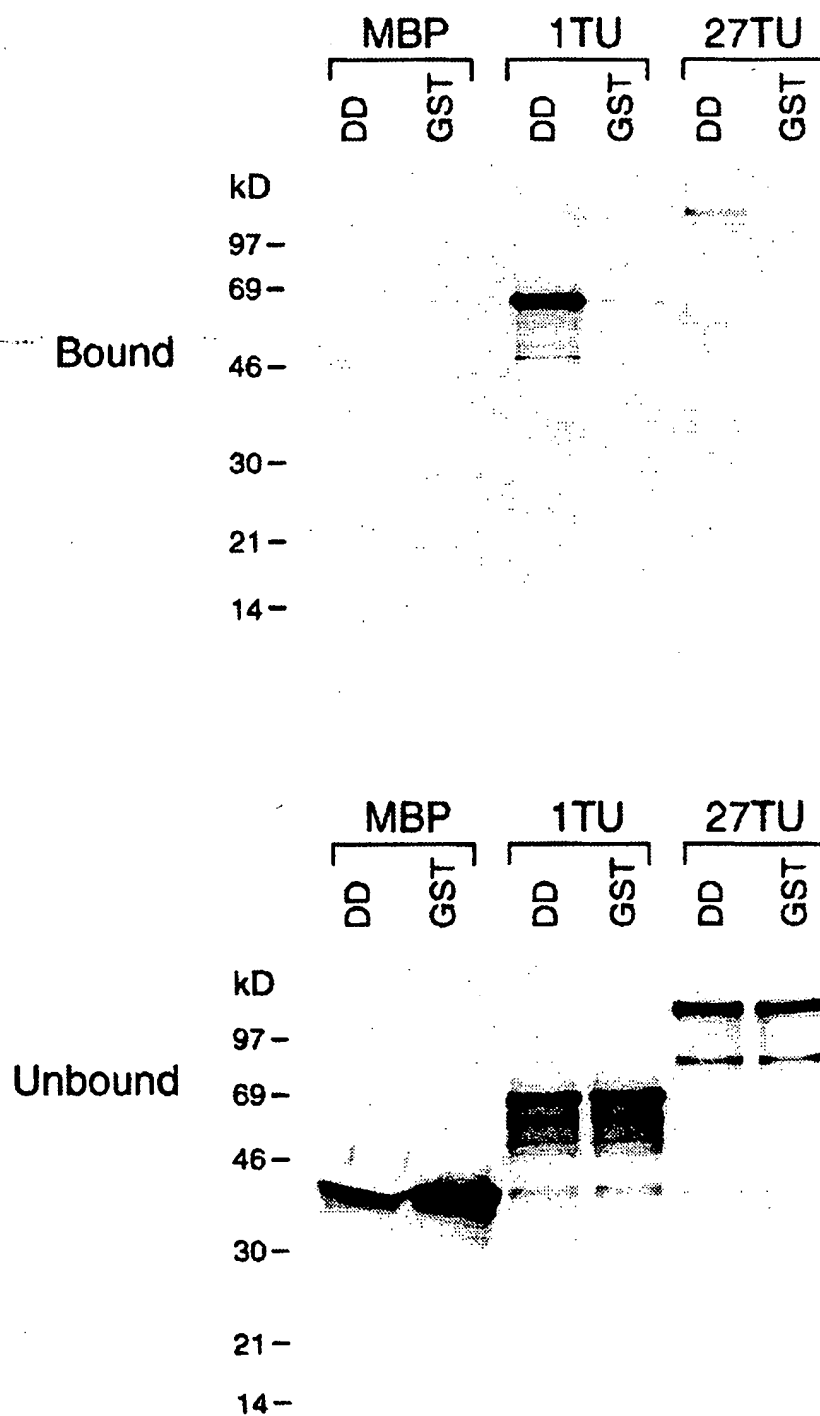


Fig. 5

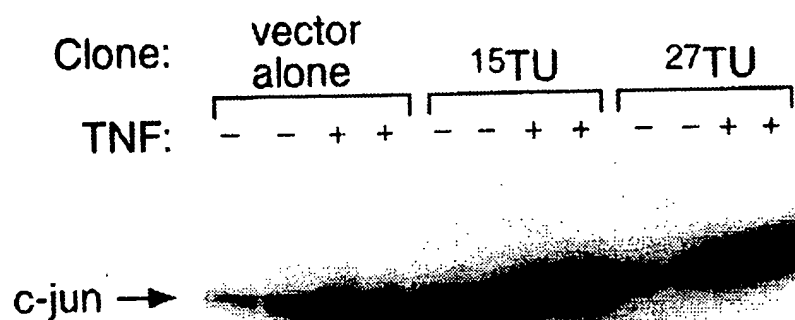


Fig. 6

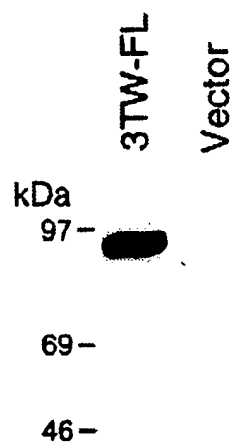
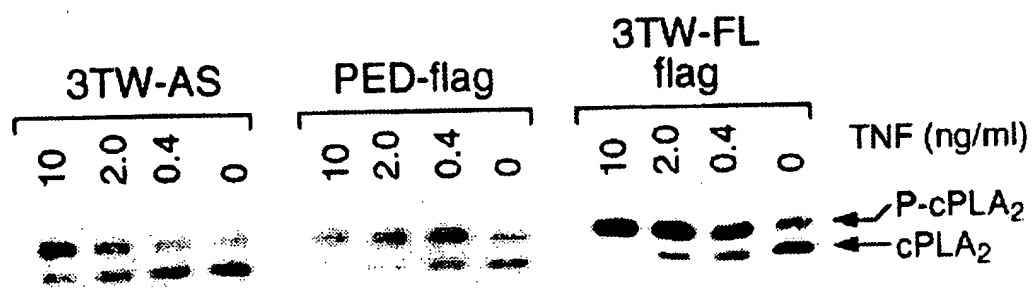


Fig. 7



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/12724

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07K14/47 C07K16/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages.	Relevance to claim No.
X	WO,A,94 01548 (MEDICAL RESEARCH COUNCIL) 20 January 1994 see sequence 494 and passim ---	1,3-8, 26-28,35
X	CELL, vol. 78, 26 August 1994 NA US, pages 681-692, M ROTHE ET AL. 'A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor' see the whole document --- -/-	1,4-8

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

'Z' document member of the same patent family

Date of the actual completion of the international search

13 February 1996

Date of mailing of the international search report

15. 03. 96

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Authorized officer

Masturzo, P

INTERNATIONAL SEARCH REPORT

International Application No.

PCI/US 95/12724

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 36, 9 August 1994 MD US, pages 22492-22495, H Y SONG ET AL. 'Aggregation of the intracellular domain of the type 1 tumor necrosis factor receptor defined by the two-hybrid system' see the whole document ---	1,4-8
A	CELL, vol. 74, no. 8, 10 September 1993 NA US, pages 845-853, L A TARTAGLIA ET AL. 'A novel domain within the 55 kD TNF receptor signals cell death' cited in the application see the whole document ---	1
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 1, 6 January 1995 MD US, pages 387-391, M P BOLDIN ET AL. 'Self-association of the "Death-Domains" of the p55 Tumor Necrosis factor (TNF) receptor and Fas/Apo1 prompts signalling for TNF and Fas/Apo1 effects' see the whole document ---	1
P,X	CELL, vol. 81, 19 May 1995 NA US, pages 495-504, H HSU ET AL. 'The TNF receptor 1-associated protein TRADD signals cell death and NF-kappaB association' see the whole document ---	1
P,X	FEBS LETTERS, vol. 367, 1995 AMSTERDAM NL, pages 39-44, M P BOLDIN ET AL. 'A protein related to a proteasome subunit binds to the intracellular domain of the p55 TNF receptor upstream to its "death domain" see the whole document ---	1
P,X	PROTEIN ENGINEERING, vol. 8 supplement, 1995 ENGLAND GB, page 90 MMY WAYE ET AL. 'Gene expression of adult human heart as revealed by random sequencing of cDNA library' see the whole document ---	1

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 95/12724

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	COMPTES RENDUS DES SEANCES DE L'ACADEMIE DES SCIENCES SERIE III: SCIENCES DE LA VIE., vol. 318, 1995 MONTREUIL FR, pages 263-272. C AUFFRAY ET AL. 'IMAGE: intégration au niveau moléculaire de l'analyse du génome humain et de son expression' see the whole document ---	1
X	WO,A,94 10207 (CHIRON) 11 May 1994 see the whole document ---	1,11, 13-17, 19-28
X	WO,A,92 14834 (WHITTIER INSTITUTE) 3 September 1992 see the whole document ---	1,11, 13-17, 19-28
X	WO,A,92 03471 (CHIRON) 5 March 1992 see the whole document ---	1,11, 13-17, 19-28
X	WO,A,92 03470 (CHIRON) 5 March 1992 see the whole document -----	1,11, 13-17, 19-28

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/12724

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 16, 18-20, 23-24
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims refer to a method of treatment of the human body, the search was carried out and based on the alleged effects of the products.
See also continuation sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US95/ 12724

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

No other distinguishing feature has been provided for the problems of claim 1 than their ability to bind the death domain of the TNF-R1 receptor.

This makes a complete search impossible for economical reasons.

The search was limited to real examples (seq. 1-16) provided by the applicant.

Claims searched incompletely: 1,4-8,12-14,16-18,21-24,26,28

INTERNATIONAL SEARCH REPORT

(Information on patent family members)

International Application No

PCT/US 95/12724

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9401548	20-01-94	AU-B- 4512193 EP-A- 0587279	31-01-94 16-03-94
WO-A-9410207	11-05-94	AU-B- 5665294 CA-A- 2148685 EP-A- 0668879	24-05-94 11-05-94 30-08-95
WO-A-9214834	03-09-92	NONE	
WO-A-9203471	05-03-92	CA-A- 2090702 CA-A- 2090703 CA-A- 2090704 CA-A- 2090705 EP-A- 0546053 EP-A- 0546074 EP-A- 0546081 EP-A- 0546110 JP-T- 6503709 JP-T- 6503947 JP-T- 6503711 JP-T- 6503949 WO-A- 9203469 WO-A- 9203152 WO-A- 9203470 US-A- 5212074	01-03-92 01-03-92 01-03-92 01-03-92 16-06-93 16-06-93 16-06-93 16-06-93 28-04-94 12-05-94 28-04-94 12-05-94 05-03-92 05-03-92 05-03-92 18-05-93
WO-A-9203470	05-03-92	CA-A- 2090702 CA-A- 2090703 CA-A- 2090704 CA-A- 2090705 EP-A- 0546053 EP-A- 0546074 EP-A- 0546081 EP-A- 0546110 JP-T- 6503709 JP-T- 6503947 JP-T- 6503711 JP-T- 6503949 WO-A- 9203469 WO-A- 9203152	01-03-92 01-03-92 01-03-92 01-03-92 16-06-93 16-06-93 16-06-93 16-06-93 28-04-94 12-05-94 28-04-94 12-05-94 05-03-92 05-03-92

information on patent family members

PCT,US 95/12724

Form PCT/ISA/210 (patent family annex) (July 1992)

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